

Ruminant models of prenatal growth restriction

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Intrauterine growth restriction (IUGR) is a significant health issue that not only affects infant mortality and morbidity, but may also predispose individuals to coronary heart disease, diabetes, hypertension and stroke as adults. The majority of IUGR pregnancies in humans are characterized by asymmetric fetal growth, resulting from inadequate nutrient transfer to the fetus. Furthermore, most of these pregnancies involve functional placental insufficiency, and may also show altered umbilical velocimetry. As the severity of IUGR increases, the fetus becomes increasingly hypoxic, hypoglycaemic and acidotic. In addition, placental transfer or utilization of some amino acids is known to be altered in IUGR pregnancies. Although a great deal has been learned from clinical studies of human IUGR, appropriate animal models are required to define completely the mechanisms involved in the development of IUGR. The pregnant sheep is a long-standing model for placental–fetal interactions, and fetal growth restriction can be induced in pregnant sheep by maternal nutrient restriction, maternal nutrient excess, administration of glucocorticoid, utero–placental embolization, carunclectomy and maternal hyperthermia. Although all of these sheep models are capable of inducing fetal growth restriction, the degree of restriction is variable. This review compares these sheep models of IUGR with the characteristics of human IUGR.

Introduction

The increase in human infant mortality and morbidity resulting from intrauterine growth restricted (IUGR) pregnancies is a significant human health issue, but there is now compelling evidence that IUGR predisposes individuals to coronary heart disease, diabetes, hypertension and stroke (Barker, 1998). The realization that alterations in the *in utero* environment result in 'fetal programming' and ultimately contribute to the onset of adult disease has led to resurgence in fetal growth research. Although the short- and long-term complications associated

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with human IUGR provide the impetus for this research, only so much can be learned from human clinical studies, thereby predicating the need for animal models of IUGR.

Of the species used for IUGR studies, the pregnant sheep provides several advantages as an animal model to examine the development and progression of IUGR pregnancies, not the least of which is the ability to cannulate both mother and fetus for nutrient transfer and utilization studies. Fetal growth restriction in sheep can be induced by nutrient restriction, nutrient excess, administration of glucocorticoids, utero-placental embolization or uterine artery ligation, limitation of placental implantation sites (carunclectomy) and exposure to increased ambient temperatures. The degree of fetal growth restriction varies among these models, as do the mechanisms involved in generating fetal growth restriction. This variation becomes important when evaluating a particular protocol, especially when it is being used as a model of human IUGR. The purpose of this review is to make such comparisons, in relation to what is known about human IUGR. In this review, it is not feasible to include a discussion of all studies, but instead we have attempted to select representative information derived from the various models for our comparisons.

Intrauterine growth restriction in humans

Intrauterine growth restriction signifies an infant that has not achieved its optimal growth *in utero*, whereas the designation 'small for gestational age (SGA)' is a statistical inference based on a given population and, therefore, the terms are not synonymous. In essence, IUGR is a clinical assessment and although many SGA infants are classified as IUGR, many are not, and an IUGR infant may not fall within the SGA designation. IUGR fetuses are categorized as having either 'symmetrical' or 'asymmetrical' patterns of growth. Symmetrical growth restriction is more rare, typically resulting from genetic abnormalities or infections that directly affect the fetus during the first trimester, providing a more uniform reduction in the size of all organs. Asymmetrical growth restriction is often associated with maternal or placental factors that result in reduced nutrient delivery to the fetus, especially during late gestation when absolute growth of the fetus is at its greatest. Consequently, many abdominal organs, especially the liver, are reduced in size, whereas the circumference of the head is maintained. This asymmetrical growth pattern is inferred from a low ponderal index ($PI = \text{weight (g)}/\text{crown-rump length (cm}^3) \times 100$) following delivery, but this index is not readily determined *in utero*.

A difficulty encountered when trying to define the mechanisms responsible for human IUGR is that not all IUGR pregnancies have the same degree of severity (Pardi *et al.*, 1993), and the type or severity of IUGR being investigated is not always clear. The majority of human IUGR pregnancies present an asymmetric growth pattern, and most of these are associated with abnormalities in placental structure and function. It stands to reason that unless there is maternal malnutrition, if a fetus is malnourished leading to asymmetric IUGR, the placenta is failing to provide adequate nutrient transfer. Accordingly, considerable effort has been placed on understanding the aetiology of placental insufficiency. Kingdom and Kaufmann (1997) suggested three distinct types of fetoplacental hypoxia that could be associated with the different types of IUGR. These include: (i) pre-placental hypoxia, in which there is uniform hypoxia within the mother, placenta and fetus, as may occur at high altitude or as a result of maternal asthma or anaemia; (ii) uteroplacental hypoxia, in which maternal oxygenation is normal but impaired placental circulation results in placental and fetal hypoxia, as in pre-eclampsia; and (iii) post-placental hypoxia, in which only the fetus is believed to be hypoxic. Another way of classifying these IUGR pregnancies is early or late onset IUGR (Kingdom *et al.*, 2000). Early onset IUGR is the most severe and encompasses the

Table 1. The effect of intrauterine growth restriction (IUGR) in humans and sheep on placental and fetal mass, fetal oxygenation and glycaemia

Model/timing	Placental mass (g)	Fetal mass (g)	Fetal oxygenation	Fetal glycaemia
Human IUGR pregnancies	↓	↓ 32–48%	↓ 33–38%	↓
Ovine experimental models				
Maternal undernutrition				
Early to mid-gestation	↔	↔	?	?
Early to late gestation	↓ 15–25%	↓ 22–45%	?	?
Late gestation	↓ or ↔	↓ or ↔	↔	↓ 63%
Maternal overnutrition				
Early to late gestation	↓ 36–45%	↓ 28–38%	↓ 30%	↓ 21%
Glucocorticoid administration				
Late gestation	↓ 25%	↓ 17–31%	↔	↔
Placental embolization				
Late gestation	↓ 27–49%	↓ 15–66%	↓ 14–44%	↓ 0–50%
Carunclectomy				
Early to late gestation	↓ 51–58%	↓ 31–37%	↓ 24%	↓ 34%
Maternal hyperthermia				
Early to late gestation	↓ 58–64%	↓ 47–59%	↓ 22%	↓ 37%
Mid- to late gestation	↓ 53–58%	↓ 17–27%	?	?

↔ No increase or decrease from control values.

post-placental hypoxia group. In late onset IUGR placenta, the lower oxygen tension within the placenta promotes a shift towards branching angiogenesis (Kingdom *et al.*, 2000) within the placental villi, whereas in early onset IUGR (post-placental hypoxia) a shift towards non-branching angiogenesis is believed to occur. In either case, altered placental villi angiogenesis may alter the oxygen and nutrient transfer capacity of the placenta and undoubtedly alters placental perfusion. Doppler velocimetry measurements appear to support these conclusions (Kingdom *et al.*, 2000), as end diastolic flow in the umbilical arteries is preserved within the late onset IUGR classification, but absent or reversed end diastolic flow of the umbilical arteries is observed in early onset IUGR.

The use of ultrasonography to assess Doppler waveforms in placental and fetal blood vessels is an important diagnostic tool in IUGR. Accordingly, Pardi *et al.* (1993) proposed that IUGR be classified into three groups based on severity. Group 1 (IUGR-1) exhibits normal fetal heart rate and a normal pulsatility index of the umbilical artery, normal fetal oxygenation and acid–base balance. IUGR-2 pregnancies have normal fetal heart rate, abnormal pulsatility index of the umbilical artery, and an increasing incidence of lactic acidosis and hypoxia. The most severe cases (IUGR-3) present as having abnormal fetal heart rate, an abnormal umbilical artery pulsatility index, and the majority of the fetuses are acidotic and hypoxic. This IUGR-3 group is comparable to the early onset classification of Kingdom *et al.* (2000). Other characteristics of human IUGR, predominantly derived from IUGR-3 pregnancies, are summarized (Tables 1–3), and provide the basis for comparisons among the sheep models of IUGR.

Pardi *et al.* (1993) and Marconi *et al.* (1996) reported that, as the severity of IUGR increases, the degree of fetal hypoxia and hypoglycaemia also increases. Although it is unclear whether blood flow to the IUGR uterus (that is, uterine artery blood flow) is altered, umbilical vein flow (Table 2) is reduced in IUGR pregnancies (Ferrazzi *et al.*, 2000; Rigano *et al.*, 2001),

Table 2. The effect of intrauterine growth restriction (IUGR) in humans and sheep on absolute uterine artery and umbilical vein blood flow, and umbilical vein blood flow normalized for fetal mass

Model/timing	Uterine artery blood flow (ml min ⁻¹)	Umbilical vein blood flow (ml min ⁻¹)	Umbilical vein blood flow (ml min ⁻¹ kg ⁻¹)
Human IUGR pregnancies	?	↓ 28%	↓ 45–49% ^a
Ovine experimental models			
Maternal undernutrition			
Early to mid-gestation	?	?	?
Late gestation	↓ 30%	↔	?
Maternal overnutrition			
Early to late gestation	↓ 42%	↓ 47%	↓ 15%
Glucocorticoid administration			
Late gestation	?	?	?
Placental embolization			
Late gestation	↓ 27–45%	↓ 21–33%	↓ 0–39%
Carunclectomy			
Early to late gestation	↓ 60%	↓ 43%	↔
Maternal hyperthermia			
Early to late gestation	↓ 33–58%	↓ 52–62%	↓ 27%

^aFetal mass estimated from ultrasound measurements of abdominal circumference (Ferrazzi *et al.*, 2000; Rigano *et al.*, 2001).

↔ No increase or decrease from control values.

Table 3. The effect of IUGR in humans and sheep on umbilical vein (UmbV) oxygen, glucose and amino acid uptake

Model/timing	UmbV O ₂ uptake	UmbV glucose uptake	UmbV amino acid uptake
Human IUGR pregnancies	↓	?	↓ 30%
Ovine experimental models			
Maternal undernutrition			
Early to mid-gestation	?	?	?
Late gestation	↔	↓ 54%	?
Maternal overnutrition			
Early to late gestation	↓ 25%	↓ 35%	?
Glucocorticoid administration			
Late gestation	?	?	?
Placental embolization			
Early to late gestation	↓ 21%	↓ 29%	?
Carunclectomy			
Early to late gestation	↓ 59%	↓ 42%	?
Maternal hyperthermia			
Early to late gestation	↓ 59%	↓ 48–60%	↓ 27–69%

↔ No increase or decrease from control values.

when expressed in either absolute terms (ml min⁻¹) or when normalized on fetal size (ml min⁻¹ kg⁻¹). This reduction in umbilical vein flow appears to be coupled with a reduction in uterine O₂ extraction (Pardi *et al.*, 1992), such that the maternal:fetal O₂ gradient is increased, implying that either the reduction in placental size or a more direct impairment of

O₂ diffusion is setting the stage for fetal hypoxia. A similar scenario may also occur with glucose transfer across the IUGR placenta, in that as the severity of IUGR increases, the degree of fetal hypoglycaemia increases as does the maternal:fetal glucose gradient (Marconi *et al.*, 1996). The change in glucose uptake of the umbilical vein (Table 3) is not known, and data from studies *in vitro* indicate that there is no change in placental glucose transporters (Jansson *et al.*, 1993), such that the reduction in fetal glycaemia may result from reductions in placental size or metabolic adaptations by the fetus. However, placental transport capacity of glucose *in vivo* has not been fully investigated in human IUGR, and animal models offer an opportunity to gain further insight. In contrast to O₂ and glucose, which traverse the placenta by passive or facilitated diffusion, amino acid transfer requires active transport mechanisms. In human IUGR pregnancies, reductions (Table 3) in fetal amino acid concentrations, placental transport and the system A transporter occur; and the transporter system L and that for taurine are also altered (Glazier *et al.*, 1997; Jansson *et al.*, 1998; Marconi *et al.*, 1999; Paolini *et al.*, 2001). Although the transplacental flux of leucine and phenylalanine is reduced in IUGR pregnancies (Paolini *et al.*, 2001), this is not the case for glycine and proline, which may infer that specific transporter systems are affected more than other systems.

Intrauterine growth restriction in sheep

Although the nutrient transfer studies in human IUGR pregnancies *in vivo* have provided considerable evidence that placental function is altered, especially in the more severe IUGR pregnancies, there is a limitation to what can be answered by these studies. Therefore, animal models, such as those that have been developed in sheep, are needed to address the mechanistic questions related to the development of IUGR. Some of the sheep models of IUGR closely mimic what is known about human IUGR.

Maternal undernutrition

The ability to restrict growth of sheep fetuses by maternal undernutrition throughout most of pregnancy was clearly defined by Wallace (1948), and has subsequently been examined under a variety of experimental paradigms. However, renewed interest in the impact of maternal undernutrition resulted from human epidemiological studies of the 'Dutch famine' (Roseboom *et al.*, 2001), and the implications that maternal undernutrition may help 'program' the offspring for adult disease. For our purposes, this cohort is interesting in that fetal growth restriction occurred only if nutrient restriction was during mid- or late gestation, yet restriction at any time during gestation had long-term ramifications on the health and well-being of the offspring (Roseboom *et al.*, 2001). Nutrient restriction from early to mid-gestation (approximately day 30 to day 80; duration of gestation approximately 150 days) in sheep does not result (Table 1) in fetal growth restriction or a reduction in placental mass near term (Steyn *et al.*, 2001), and at least in one case, there was a significant increase in placental mass (Heasman *et al.*, 1998). However, as mentioned earlier, nutrient restriction from early to late gestation (Mellor and Murray, 1982; Wallace, 1948) results in significant reductions in both fetal and placental mass (Table 1). Nutrient restriction during late gestation only may or may not result (Table 1) in placental or fetal growth restriction (Mellor, 1983; Chandler *et al.*, 1985), the results of which may be dependent on maternal nutrient 'reserves' at the onset of restriction. Taken together these data indicate that nutrient restriction from mid- to late gestation probably affects the fetus directly, and that nutrient restriction during maximum placental development (early to mid-gestation) does not result in functional placental insufficiency.

Although nutrient restriction from early to mid-gestation does not result in fetal growth restriction and, therefore, does not truly model human IUGR, these studies are important in developing an understanding of 'fetal programming' (Barker, 1998). Unfortunately, these studies have not addressed fetal oxygenation or glycaemia (Table 1), uterine or umbilical blood flows (Table 2), or umbilical uptake of O₂, glucose or amino acids (Table 3). Such experimental endpoints may not seem important if the question at hand is not fetal growth restriction, but alterations of these parameters could be directly involved in the 'fetal programming' phenomenon and should be considered. The data of Hoet and Hanson (1999) highlight this point, in that maternal undernutrition during the first 30 days of gestation results in fetal hypotension during late gestation and hypertension during postnatal life.

Maternal overnutrition

Intuitively, excess maternal nutrition would not be thought to result in IUGR; however, overnourishing singleton-bearing adolescent sheep throughout gestation results (Table 1) in significant reductions in placental mass and fetal mass at term (Wallace *et al.*, 1999a). It appears that in overnourished adolescent ewes, maintenance of maternal growth becomes the priority at the expense of providing adequate nutrient supply to the gravid uterus. Interestingly, if maternal dietary intake is reduced from the high level to a moderate level at day 50 of gestation, placental and fetal growth is enhanced (Wallace *et al.*, 1999b). In contrast, at the beginning of mid-gestation (day 50), shifting from a moderate diet to the high diet results in a reduction in placental and fetal growth. These later data indicate that early gestational effects are at least in part reversible (Wallace *et al.*, 1999b). In continuously overnourished pregnancies (Wallace *et al.*, 2002a), the fetus becomes hypoxic and hypoglycaemic during late gestation (Table 1), similar to human IUGR. This appears to be a function of reductions in uterine arterial and umbilical venous flows (Table 2), coupled with reductions in umbilical venous O₂ and glucose uptake (Table 3) (Wallace *et al.*, 2002a). Potential alterations in umbilical amino acid uptake have not been reported. Again, these data indicate that, in many ways, the overnourished adolescent ewe model recapitulates what is known about human IUGR. However, it is not known whether the changes in uterine-umbilical blood flows and umbilical nutrient uptake result from altered placental vascularity or resistance, as assessed by umbilical artery Doppler velocimetry. In other words, the changes observed could be strictly related to placental and fetal size, rather than to changes in placental-fetal haemodynamics, as measured by end diastolic flow in the umbilical artery (Pardi *et al.*, 1993; Kingdom *et al.*, 2000). This possibility is supported by recent glycaemic clamp studies that indicate that the small size of the placenta, rather than alterations in placental glucose uptake, metabolism or transport, is responsible for fetal hypoglycaemia (Wallace *et al.*, 2002b).

Administration of glucocorticoid

One of the adjustments made by the fetus in response to a lack of nutrients is a change in its endocrine environment, including an increase in circulating cortisol concentrations (Barker, 1998). Increasing concentrations of cortisol in fetal circulation during late gestation are believed to be involved in the maturation of some organ systems. Antenatal administration of synthetic glucocorticoids to women at risk of preterm delivery is a common clinical practice to stimulate fetal lung maturity. However, there is considerable evidence that antenatal exposure to glucocorticoids reduces birth weight and results in lifelong hypertension, hyperglycaemia and hyperinsulinaemia (Seckl, 2001). Maternal administration of glucocorticoids during late gestation in sheep (Table 1) reduces fetal growth (Jobe *et al.*, 1998; Moss *et al.*, 2001) and, at least in one report, resulted in reduced placental size (Jensen *et al.*, 2002). Fetal blood gases

are not altered after maternal glucocorticoid administration (Sloboda *et al.*, 2000), nor are fetal glucose concentrations (Jensen *et al.*, 2002), but fetal blood pressure was increased by 30% (Jensen *et al.*, 2002). Uterine or umbilical blood flow or placental transport of essential nutrients have not been evaluated in these experimental paradigms (Tables 2 and 3), but they have been evaluated after glucocorticoid induced parturition. From these and other studies it may be inferred that prenatal exposure to glucocorticoids affects fetal growth directly, rather than through alterations in placental function and nutrient transfer. This conclusion is corroborated by the fact that maternal administration of glucocorticoids at day 27 of gestation does not affect placental or fetal growth, but does result in adult hypertension of these offspring (Dodic *et al.*, 2002). Although maternal administration of glucocorticoids is a popular and useful approach for initiating aspects of 'fetal programming,' it is difficult to interpret fully these studies in context with human IUGR, as functional placental insufficiency may not be a hallmark of this model. However, these studies may have application to the increased stress observed in pre-eclamptic pregnancies.

Utero-placental embolism

Utero-placental embolism (UPE) is achieved through: (i) restriction of uterine blood perfusion or (ii) restriction of umbilical placental perfusion, by infusion of microspheres, vessel occlusion or ligation of a single uterine artery during late gestation. Subsequent reductions (Table 1) in fetal mass range from 15% (Lang *et al.*, 2000) to 66% (Oyama *et al.*, 1992) and placental mass reductions range from 27 to 49%. These fetuses are hypoxic (Clapp *et al.*, 1981) and hypoglycaemic (Louey *et al.*, 2000), although there is considerable variation among reports. Inherent to this model, uterine artery and umbilical artery blood flows (Table 2) are reduced, but the amount of reduction is variable (Block *et al.*, 1990; Lang *et al.*, 2000). This variability may be attributable to variation in gestational age, degree of embolism achieved and site of embolism or occlusion. Metabolic status (Table 3) of the fetus and placenta is not clearly understood in the UPE model, as only Clapp *et al.* (1981) assessed uptake of oxygen and glucose by the fetal circulation. Fetal uptake of both O₂ and glucose are reduced after UPE (Table 3), but unfortunately transplacental fluxes of amino acids have not been reported. On the basis of the data presented, the UPE model appears closely to recapitulate the events that occur in human IUGR, including fetal hypertension (Louey *et al.*, 2000). The primary shortcoming of this model is that the changes in utero-placental blood flow are artificial and part of the model, rather than having resulted from the placenta developing functional insufficiency itself. In other words, although this model is excellent for examining the impact of reduced utero-placental blood flow on fetal growth, a major factor in the development of human IUGR, that is, placental function, is dependent on the experimental paradigm.

Carunclectomy

A long-standing method for generating fetal growth restriction in sheep is the surgical removal of placental attachment sites, uterine caruncles, before mating (Alexander, 1964). In doing so, both placental and fetal mass are significantly reduced (Table 1) near term (Owens *et al.*, 1987, 1989). However, not all pregnancies result in fetal growth restriction (Owens *et al.*, 1987), indicating that in about half of these pregnancies the placenta is able to compensate functionally. When IUGR ensues, as gestation advances, the fetus becomes hypoxic (Owens *et al.*, 1987) and hypoglycaemic (Owens *et al.*, 1989). Fetal hypoxia and hypoglycaemia appear to result from a reduction in umbilical uptake of O₂ and glucose (Table 3), coupled with reductions (Table 2) in uterine and umbilical blood flows (Owens

et al., 1987; 1989). It is notable that although umbilical glucose uptake ($\mu\text{mol min}^{-1}$) was significantly less in carunclectomized IUGR fetuses, uptake on a fetal mass basis ($\mu\text{mol min}^{-1} \text{kg}^{-1}$ fetus) was not different between growth restricted and control pregnancies (Owens *et al.*, 1989). In many ways these data are similar to human IUGR, and the maternal overnutrition model (Wallace *et al.*, 2002a), but without glycaemic clamp studies it is not discernible whether this results solely from placental mass reductions, or from glucose transporter deficits. Umbilical uptake of specific amino acids (Table 3) has not been reported for this model. On the basis of the parameters that this review has focused on, this model of IUGR mimics many of the hallmarks of human IUGR. However, it is interesting to note that in this model, fetal arterial blood pressure does not increase in these growth-restricted fetuses (Edwards *et al.*, 1999), whereas it does in response to maternal glucocorticoid administration and in the UPE model. Furthermore, although this may be one of the best-characterized models, the structural and functional changes within these placentae are not understood. For example, is the placental vasculature altered during placental development in these pregnancies, and how might the placentae of the growth-restricted pregnancies differ from those of the carunclectomized pregnancies that do not result in IUGR. The lack of effect on fetal arterial pressure (Edwards *et al.*, 1999) may well indicate that umbilical arterial Doppler waveforms are not altered in this IUGR model, as they are in the most severe cases of human IUGR. Nevertheless, this is a very useful model, and one that has provided considerable insight into fetal growth restriction, and is being used extensively to examine the effects of fetal programming.

Maternal hyperthermia

Growth restriction of sheep fetuses can be induced by exposing pregnant ewes to high ambient temperatures (Alexander and Williams, 1971) from early to late gestation, resulting in some of the most severe cases of IUGR. Exposure of pregnant ewes to a hyperthermic environment (Bell *et al.*, 1989; Early *et al.*, 1991) from mid- (day 64) to late gestation (days 136–141) results in reduced fetal and placental mass (Table 1), whereas exposure from early (days 33–39) to late gestation (days 112–135) results in greater reductions in fetal and placental mass (Ross *et al.*, 1996; Anderson *et al.*, 1997; Thureen *et al.*, 1992). Similar results are obtained (Galan *et al.*, 1999) when exposure is for only 55 days (days 37–93), and the growth restriction of the placenta and fetus is associated with an increase in maternal core body temperature approximating 0.8°C (Regnault *et al.*, 1999). Fetal growth in these pregnancies is asymmetric, as indicated by greater biparietal diameter:abdominal circumference ratios (Galan *et al.*, 1999) and reduced ponderal indices (Regnault *et al.*, 1999). In addition, current evidence indicates that development of IUGR is a consequence of reduction in placental growth from early to mid-gestation (Bell *et al.*, 1989; Thureen *et al.*, 1992; Galan *et al.*, 1999), making this a relevant model to examine impaired placental development, that is, placental insufficiency that results in IUGR.

Studies of this IUGR sheep model indicate that the fetus (Table 1) is hypoxic and hypoglycaemic (Thureen *et al.*, 1992; Ross *et al.*, 1996; Anderson *et al.*, 1997), resulting from a reduction in umbilical O_2 and glucose uptake (Table 3). In addition, uterine and umbilical blood flows are reduced (Table 2), as is umbilical vein flow when normalized on fetal size (Bell *et al.*, 1987). If umbilical glucose uptake is expressed on a kg of fetus basis, there is no difference between control and IUGR fetuses (Thureen *et al.*, 1992), similar to the other models already discussed. However, Thureen *et al.* (1992) conducted hypo- and hyper-glycaemic clamp studies, and determined that there was indeed reduced capacity for placental glucose transport in the hyperthermic IUGR model. Similar to human IUGR, transport of at least

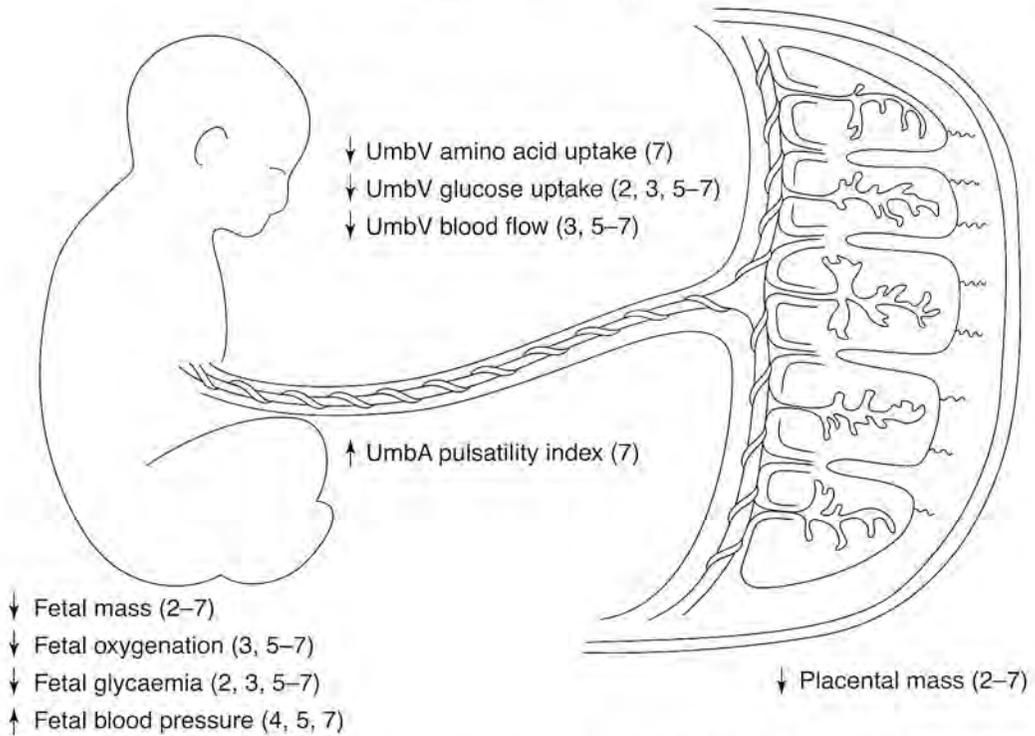


Fig. 1. Characteristics of human intrauterine growth restricted (IUGR) pregnancies. The arrows indicate changes, and the numbers in parentheses indicate which sheep model of IUGR exhibit the same change: 1: early to mid-gestation maternal undernutrition; 2: late gestation maternal undernutrition; 3: maternal overnutrition; 4: maternal glucocorticoid administration; 5: placental embolization; 6: carunclectomy; 7: maternal hyperthermia. UmbA: umbilical artery; UmbV: umbilical vein.

two essential amino acids, leucine and threonine, across the placenta (Table 3) is reduced, resulting in the routing of these two amino acids into fetal accretion and away from oxidation (Ross *et al.*, 1996; Anderson *et al.*, 1997). The reduction in transport is not simply a function of placental mass, as transport was still less when normalized on placental mass. Taken together, these data indicate that this model of IUGR in sheep recapitulates many of the hallmarks of human IUGR pregnancies.

As indicated in the discussion of human IUGR, alterations in placental angiogenesis have been associated with early versus late onset IUGR (Kingdom *et al.*, 2000). Expression of a variety of angiogenic growth factors and their respective receptors is altered in hyperthermia-induced IUGR placenta (Regnault *et al.*, 2002a,b), resulting in changes in the placental vascular architecture (Regnault *et al.*, 2002a), changes in umbilical Doppler velocity waveforms (Galan *et al.*, 1998) and increased fetal arterial blood pressure (H. L. Galan, T. R. H. Regnault and R. V. Anthony, unpublished), similar to that reported in humans. The variations in placental vascular architecture, placental angiogenesis, uterine and umbilical blood flows, and placental vascular resistance (Regnault *et al.*, 2002a) occur in concert with impaired O_2 , glucose and amino acid transfer to the fetus, setting the stage for asymmetric fetal growth restriction. The impaired ability of a simple nutrient, such as O_2 , to cross the placenta (Regnault *et al.*, 2002a) in this model of IUGR exemplifies how important normal placental development and function is to fetal growth.

Conclusions

In this review, we have highlighted common features shown in human IUGR pregnancies with those that have been examined in several sheep models of IUGR. All of these models have strengths and weaknesses, and no animal model will fully recapitulate the events that occur within a given type of human IUGR. Accordingly, it is important to measure in our sheep models the same parameters that have been measured clinically in humans to validate these models further. Figure 1 summarizes the hallmarks of human IUGR, and identifies the sheep models of IUGR that have similar characteristics. The majority of cases of human IUGR are associated with placental insufficiency, and several of the models discussed create placental insufficiency, whereas others do not. Even in sheep models that do not alter placental size, it is important to consider that placental function could still be impacted, and that placental structure and function are continually changing throughout pregnancy. Finally, considerable emphasis recently has been placed on characterizing the postnatal sequelae of IUGR in humans and in animal models. Although this is very important, we should not forget about carefully characterizing the placental–fetal interactions that result in ‘fetal programming’.

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