Developmental programming of the ovine placenta

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The pattern of intrauterine growth and size at birth, in particular, programmes the structure and function of tissues later in life in many species, which has important implications for the incidence of adult-onset generative diseases in human populations. In mammals, the main determinant of intrauterine growth is the placental supply of nutrients which, in turn, depends on the size, morphology, transport characteristics and endocrine function of the placenta. However, compared to somatic tissues, little is known about the developmental programming of the placenta. This review examines the epigenetic regulation of placental phenotype with particular emphasis on the nutrient transfer capacity of the ovine placenta and environmental factors shown to cause developmental programming of other tissues. Overall, the placenta is responsive to environmental factors and uses a number of different strategies to adapt its phenotype to help support fetal growth during adverse intrauterine conditions. It is, therefore, not just a passive conduit for nutrient transfer to the fetus but alters its nutrient supply capacity dynamically to optimise fetal nutrient acquisition. Thus, the placental epigenome provides both a memory of environmental conditions experienced during development and an index of the future well being of the offspring.

Introduction

Size at birth is critical in determining life expectancy. In human populations, the smaller the neonate the less likely it is to survive at birth and more likely it is to develop adult onset, life threatening diseases, such as hypertension, coronary heart disease and Type 2 diabetes (Barker, 1994). Similarly, in domesticated species including ruminants, natural and experimental restriction of fetal growth leads to poor neonatal viability and a failure to thrive postnatally (Greenwood & Bell 2003). Low birth weight in these animals is also associated with abnormalities in metabolic, endocrine, reproductive and cardiovascular function in later life (McMillen & Robinson 2005). Together, the epidemiological and experimental observations have led to the concept that conditions experienced *in utero* lead to a specific epigenotype with phenotypic consequences long after birth. The process by which environmental conditions during early life permanently alter tissue structure and function is known as developmental programming.

In mammals, the main determinant of size at birth is the placental supply of nutrients for fetal growth. In turn, this depends on the size, morphology, transport characteristics and

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endocrine function of the placenta (Sibley *et al.* 2005; Fowden *et al.* 2006b). Experimental manipulation of placental growth in sheep and other species leads to altered fetal growth and postnatal physiological abnormalities, consistent with the human epidemiological findings (Fowden *et al.*, 2008). Epigenetic regulation of placental phenotype may, therefore, be an important mechanism by which environmental conditions programme intrauterine development. Indeed, recent human studies have shown that the adverse cardiovascular consequences of low birth weight are related to the shape and size of the placenta at birth (Barker *et al.* 2010). However, compared to somatic tissues (McMillen & Robinson 2005; Gluckman *et al.* 2009), little is known about the programming of the placenta *per se*. This review examines epigenetic regulation of placental phenotype with particular emphasis on the nutrient transfer capacity of the ovine placenta and environmental factors shown experimentally to cause developmental programming of other tissues.

Placental size and morphology

Placental size directly affects the capacity for nutrient transfer via changes in the trophoblast surface area for transport and, when measured as placental weight, is directly related to fetal body weight at term in many species including ruminants (Baur 1977; Mellor 1983). In sheep, direct experimental restriction of placental growth by removal of implantation sites, multiple pregnancy or by embryo transfer between breeds of different sizes reduces fetal weight (Owens *et al.* 1987; Dwyer *et al.* 2005; Reynolds *et al.* 2005). Placental weight and, hence, birth weight is also determined by parity of the ewe (Dwyer *et al.* 2005). In addition, placental weight at term is affected by a wide range of environmental factors, although their specific effects depend on the severity, duration and gestational age at the onset of the perturbation (Table 1).

Both under- and over-nutrition affect placental weight at term (Table 1). Periconceptual undernutrition from 60 days before conception up to implantation at $\approx$30 days of pregnancy appears to have little effect on placental growth but, when the period of undernutrition occurs during the main period of placental growth from 40-75 days, placental weight is often increased at term (Table 1). If the period of nutrient deprivation extends into mid to late gestation, placental weight is generally lower than normal at term but not if undernutrition is confined solely to late gestation (Table 1). In addition, the body condition score of the ewe at conception, an index of pre-pregnancy nutritional state, and genetic adaptation to poor nutritional conditions can alter the placental response to subsequent undernutrition, particularly during mid gestation (Kelly 1992; Osgerby *et al.* 2003; Vonnahme *et al.* 2006). In contrast, over-nutrition for most of gestation leads to placental growth restriction at term (Table 1), especially in growing adolescents (Redmer *et al.* 2004).

Ovine placental weight appears less sensitive to changes in fetal and maternal hormone concentrations. Variations in maternal growth hormone (GH), IGF-1 and insulin levels during mid or late gestation appear to have little effect on the weight of the total placenta or of individual placentomes (DiGiacomo & Hay 1989; Harding *et al.* 1997; Wallace *et al.* 2006; Wright *et al.* 2008). Similarly, manipulating fetal hormone concentrations by exogenous infusion or endocrine gland ablation has shown that fetal pituitary, thyroid and adrenal hormones have little effect on total placental weight at term, although they influence fetal growth (see Fowden & Forhead, 2009). Placental weight near term is also unaffected by fetal administration of leptin and IGF-1 for 5-10 days during late gestation (Bloomfield *et al.* 2002; Forhead *et al.* 2008). However, maternal administration of natural or synthetic glucocorticoids during late gestation reduces placental weight in association with fetal growth restriction (Jensen *et al.* 2002; Braun *et al.* 2007). Since glucocorticoid concentrations are altered by conditions,

such as hyperthermia, undernutrition and hypoxaemia (see Fowden & Forhead 2009), these hormones may mediate, in part, the effects of environmental stimuli on the placenta. Indeed, in natural conditions, the placental effects of many of these environmental factors are likely to be multi-factorial as conditions such hypoxaemia and hyperthermia reduce food intake in pregnant ewes (Alexander & Williams 1977; Jacobs *et al.* 1988; Regnault *et al.* 2005).

Even during adverse conditions, fetal weight is still directly related to placental weight (Wallace *et al.* 2005; Quigley *et al.* 2008). However, often, the effects of these conditions are more pronounced on the placenta than fetus (Table 1). When placental growth is restricted, placental efficiency increases as more grams of fetus are produced per gram of placenta than in normal conditions (see Fowden *et al* 2009). Greater placental efficiency is also seen with maternal glucocorticoid treatment, multiple pregnancy and increasing parity of the ewe (Jensen *et al.* 2002; Dwyer *et al.* 2005). Hardier breeds of sheep also tend to have higher placental efficiencies than breeds evolutionarily adapted to better nutritional conditions (Dwyer *et al.*, 2005). These observations suggest that the placenta can adapt to the fetal nutrient demands for growth and help maintain normal fetal growth when its own growth is compromised. These adaptations may have a morphological or functional origin.

Ovine placentomes can be classified into 4 types, A to D, using their gross morphological appearance (Vatnick *et al.* 1991). The smaller, rounder A and B type placentomes predominate throughout gestation and, on average, account for about 60% or more of the total number under normal conditions. The larger, flatter C and D type placentomes are less common but increase in frequency during late gestation, although their numbers appear to decrease again close to term (see Fowden *et al.* 2006b). In general, adverse environmental conditions during the period of maximal placental growth lead to a shift from A-type placentomes to the more everted types later in gestation (Table 1). While this shift is often associated with placental growth restriction, changes in placentome frequency distribution have been observed without any change in total placental weight in response to both environmental and hormonal stimuli (Penninga & Longo 1998; Bloomfield *et al.* 2002). This has lead to the suggestion that the presence of more C and D-type placentomes is an adaptation to increase placental efficiency and the transfer of nutrients to the fetus (Heasman *et al.* 1998; Steyn *et al.* 2001; Vonnahme *et al.* 2006). Certainly, in carunclectomized ewes with small placentas composed solely of large D-type placentomes, the rate of glucose transfer to the fetus per gram of placenta is enhanced relative to controls (Owens *et al.* 1987; 1989). However, in normal conditions during late gestation, there is little, if any, evidence for changes in placental weight, efficiency or glucose transfer with the frequency of C/D type placentomes (Figure 1A-C).

In late gestation, adverse conditions either have little effect on placentome distribution or reduce the incidence of C/D placentome types (Table 1). This may be the consequence of elevated cortisol concentrations as both maternal and fetal glucocorticoid treatment in late gestation increases the frequency of A/B type placentomes (Jensen *et al.* 2002; 2005; Ward *et al.* 2006). By tagging individual placentomes before fetal treatment, cortisol was shown to decrease, or even reverse, the normal rate of placentome eversion with increasing gestational age, consistent with the prepartum decline in C/D placentome frequency seen during the natural fetal cortisol surge (Ward *et al.* 2006; Fowden *et al.* 2006b). Since placental glucose delivery to cortisol infused fetuses per gram of placenta is higher in animals with proportionately more A/B type placentomes (Figure 1D), the cortisol-induced slowing of the progressive ontogenic shift towards more everted placentomes may be an adaptive response to help maintain the fetal nutrient supply during late gestation (Ward *et al.* 2006). Thus, the gross morphology of the ovine placenta may be functionally significant in nutrient transport when cortisol concentrations are high.

Table 1. Effects of environmental cues during pregnancy on feto-placental growth and placental morphology in sheep near term (> 130 days).

		% Control Weight			
Treatment	Period of Treatment	Placenta	Fetus	Morphological Changes	Reference
Nutrition					
Overnutrition	-89-133	87%	100%	Less C/D type placetomes	Quigley et al. 2008
	0-128	49%	63%	Reduced mean placetome weight Decreased capillary surface density	Wallace et al. 2000 Redmer et al. 2004
Hyperglycaemia	115-135	86%	98%		Aldoretta et al. 1994
Undernutrition	-89-133	73%	82%	More C/D type placetomes	Quigley et al. 2008
	-60 -30	97%	107-93%	More D type placetomes	Oliver et al. 2005; Rumball et al. 2008
	-14-70	120%	97%	Less A more D placentomes	Steyn et al. 2001
	0-90	98%	90%	Normal placetomes distribution	Luther et al. 2007
	0-130	93%	83%	Reduced caruncle capillary area density	
	28-80	160-120%	107-100%	Less A more B type placentomes Smaller placetomes across all types Increased fetal compared to placetome	Heasman et al. 1998 Dandrea et al. 2001 Gnanalingham et al. 2007
	30-96	130%	100%		McCrabb at al. 1991
	22-135	81%	88%	Less B more C&D placetomes	McMullen et al. 2004; Osgerby et al. 2002
	50-145	77%	67%	Less fetal tissue in placetomes	Alexander & Williams 1971
	91-135	100%	100%		McMullen et al. 2004
	115-144	100%	100%		Edwards & McMillen 2001
Hypoglycaemia	70-135	68%	71%		Carver & Hay 1995
	100-135	69%	72%		Aldoretta et al. 1994
	115-135	100%	100%		DiGiacomo & Hay 1989

Table 1. Contd.

Restrict placental blood flow					
Uterine	115-138	73-66%	85-68%		Lang et al. 2000
Cord constriction	125-128	80%	100%	Less D type placetomes	Gardner et al. 2002
Embolization	125-135	78%	87%		Gangon et al. 1996
Hypoxia					
High Altitude	0 –110	100%	100%	Less A more B ,C, & D placetomes	Penninga & Longo 1998;
	30- term	108%	71%	Increased capillary area	Parraquez et al. 2006
	49 -140	100%	100%	Increased vascular area Decreased number of vessels Increased coiling	Krebs et al. 1997
Hypoxic chamber	30-135	77%	79%		Jacobs et al. 1988
	120-141	89%	83%		
Hyperthermia	40 -120	49%	58%	Increased number of fetal vessels Decreased number of maternal vessels Increased coiling of fetal vessels	Regnault et al. 2002; 2003
	50-100	68%	76%		Alexander & Williams 1971
	50-145	31%	49%		
	64-138	42%	73%	Decreased protein content	Early et al. 1991
	100-145	59%	71%		Alexander & Williams 1971
Glucocorticoid Treatment					
Mother	104, 111, 118	100%	87%	Fewer BNC	Braun et al. 2007
	115 -125	100%	110%	More B type placetomes	Jensen et al. 2005
	118 -128	75%	93%	Less A type placetomes	Jensen et al. 2002
Fetus	125 -130	100%	100%	More A and less D type placetomes Lower BNC numbers	Ward et al. 2002; 2006

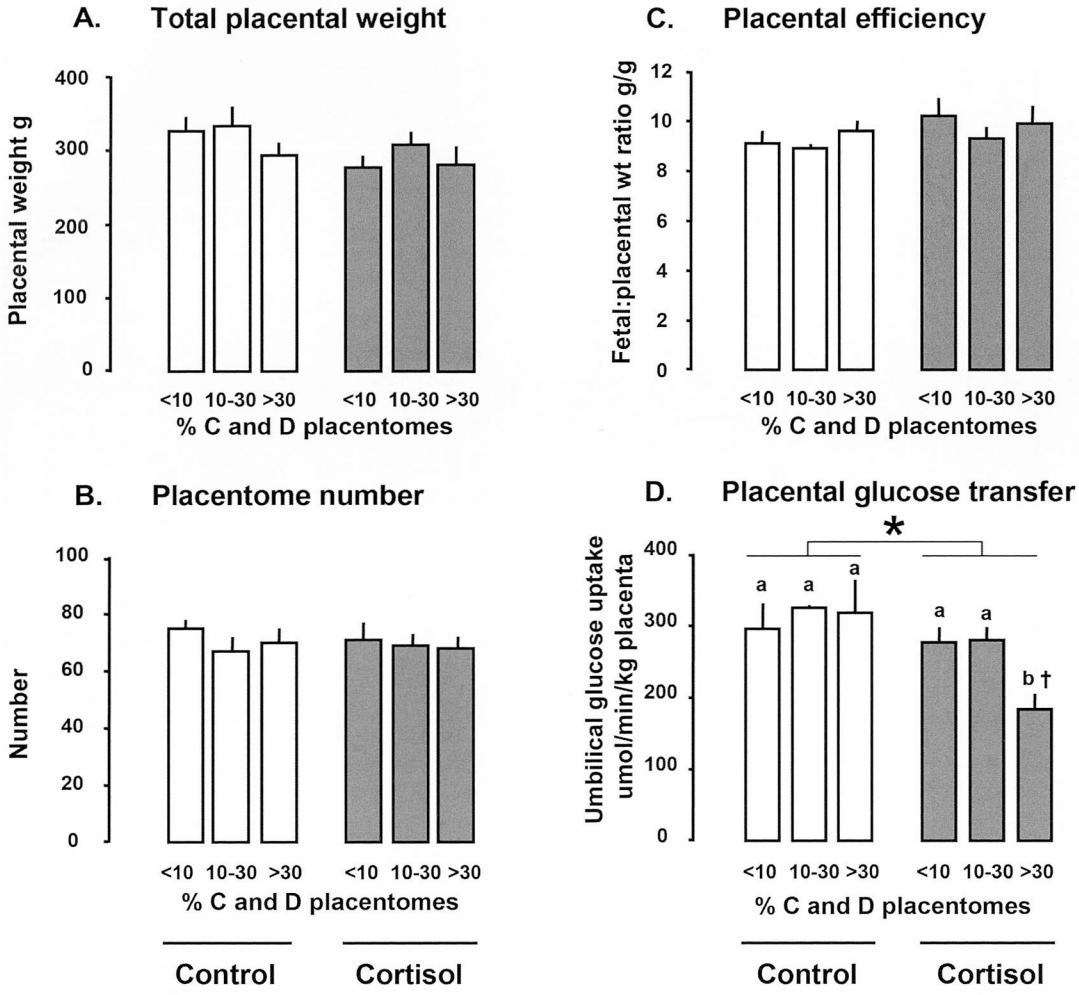

Fig. 1. Mean (± SE) values of A) total placental weight, B) placentome number, C) placental efficiency measured as gram fetus per gram placenta and D) placental glucose transfer calculated as umbilical uptake per gram placenta at the prevailing glucose concentration gradient in single sheep fetuses either infused with cortisol (1-2mg/kg fetus/day, shaded columns) or in the control state (saline infused or untreated, open columns) before delivery at 127-131 days with respect to the frequency distribution of more everted C and D type placentomes expressed as a percentage of the total placentome number (<10%, 10-30%, >30%). * significant effect of treatment $P<0.02$ two-way ANOVA. In D) within treatments, columns with different letters as superscripts are significantly different from each other $P<0.05$ (two-way ANOVA). † significantly different from respective saline infused placentome type ($P<0.05$, t-test). Number of fetuses in A), B) and C) are Controls; <10% n = 18, 10-30%, n = 8; >30%, n= 11: Cortisol; <10% n = 7, 10-30% n = 11, >30% n = 6. In D) numbers are Controls; <10% n = 6, 10-30% n = 3, >30% n = 5: Cortisol; 10% n = 5, 10-30% n = 4, >30% n = 6. Data from Gardner *et al.* 2002; Ward 2002; Ward *et al.* 2002; 2006 and Fowden, Forhead and Wooding, unpublished observations).

Much less is known about environmental influences on ovine placental ultra-structure. Glucocorticoid administration to either the ewe or fetus in late gestation prematurely decreases the number of binucleate cells (BNC) in the fetal trophectoderm (Ward *et al.* 2002; Braun *et al.* 2007), consistent with the prepartum decline in BNC numbers as fetal cortisol levels rise towards term (Wooding & Burton 2008). These cells produce placental lactogen (PL) and migrate across the maternal-fetal interface throughout pregnancy to form a syncytium by fusion with the maternal epithelium. They are, therefore, involved in placental remodelling and maternal PL delivery, both of which may influence placental efficiency (Fowden *et al.* 2009). Reduced PL concentrations have been observed in hyperthermic and overnourished ewes, although these changes probably reflect the reduced placental mass rather than decreased BNC numbers or PL content per cell (Regnault *et al.* 2005; Lea *et al.* 2007). In contrast, periconceptional undernutrition leads to raised maternal and fetal PL levels in late gestation despite normal placental weight, which suggests that BNCs can be regulated nutritionally (Oliver *et al.* 2005).

Between 50 days and term, the surface area for nutrient exchange increases in the ovine placenta along with increases in the number and/or area of the capillaries in the caruncular (maternal) and cotyledonary (fetal) portions of the placentomes (Stegmann 1975; Reynolds *et al.* 2005). The cotyledonary increments are greater due to branching angiogenesis and an increase in capillary density (Reynolds *et al.* 2010). In part, the vascular changes are driven by the fetal nutrient demands as between breed embryo transfer has shown that constraining fetal growth below its genetic potential increases placental vascularity in several species including sheep (Biensen *et al.* 1999; Allen *et al.* 2002; Reynolds *et al.* 2005). Placental vascularity also changes in response to environmental stressors (Table 1) and with gross placentome type, although not consistently across the A to D spectrum (Vonnahme *et al.* 2008). Consequently, increasing placental vascularity may explain, in part, the increased efficiency of the small placenta but does not provide a functional rationale for the shift towards more everted placentome types after adverse conditions early in development.

Both increases and decreases in the number, area and density of the placental capillaries have been observed during poor intrauterine conditions with differential responses in the cotyledonary and caruncular vasculature in some instances (Krebs *et al.* 1997; Regnault *et al.* 2002; Luther *et al.* 2007; Redmer *et al.* 2009). At high altitude, placental capillaries become more branched and looped, and their average luminal area increases in both the cotyledonary and carunclar regions (Krebs *et al.* 1997). Similar increases in vessel tortuosity have been observed in placentomes from hyperthermic ewes, in association with an increase in cotyledonary capillary number (Regnault *et al.* 2002). In addition, both over- and under-nutrition during the period of maximal placental growth affects angiogenesis with regional alterations in capillary area and/or number density, which become less pronounced with increasing gestational age (Redmer *et al.* 2004; Luther *et al.* 2007; Zhu *et al.* 2009). In many of these conditions, the changes in placental vascularity are accompanied by alterations in placental expression of various angiogenic factors (Reynolds *et al.* 2005). These include the vascular endothelial growth factor (VEGF), angiopoietin and fibroblast growth factor protein families as well as their respective receptors, all of which have several isoforms (Reynolds *et al.* 2010). By altering blood flow and surface area for exchange, these environmentally-induced changes in placental vascularity and morphology will modify nutrient transfer, particularly of lipophilic molecules, like oxygen, which cross the placenta by simple diffusion. However, to date, little is known about the epigenetic regulation of the thickness and morphology of the interhemal membrane also important in determining the passive diffusional characteristics of the placenta.

Placental transport characteristics

For facilitated diffusion and active transport, placental nutrient transport capacity depends not only on trophoblast surface area but also on expression of nutrient transporters per unit area. Transplacental diffusion of nutrients into the fetal circulation is also determined by the transplacental concentration gradient and the rate of nutrient utilisation by the utero-placental tissues themselves (Hay 2006; Fowden *et al.* 2009). All of these factors change with gestational age and during adverse intrauterine conditions (Regnault *et al.* 2005; Fowden *et al.* 2008). For example, between mid and late gestation, there are increases in the placental abundance of glucose transporter-3 (GLUT3), the transplacental glucose concentration gradient and in the relative proportion of uterine glucose uptake transfered to the sheep fetus (Fowden 1997; Hay 2006). Since some of the glucose carbon used *in utero* is passed onto the fetus as lactate (Hay 2006), changes in placental lactate production towards term and during adverse conditions will also influence the apparent glucose transfer capacity of the ovine placenta (Fowden 1997).

In late gestation, transplacental glucose transfer is altered by a range of nutritional and other perturbations, although the extent to which these changes are due to genuine alterations in the placental glucose transport capacity appears to depend on the specific insult (Table 2). For instance, the small placenta of hyperthermic ewes transports more glucose per kg placenta than controls, primarily as a result of an increased transplacental glucose concentration gradient caused by fetal hypoglycaemia (Table 2). At the normal glucose concentration gradient, the capacity for glucose transport per kg of hyperthermic placenta is actually 20-30% lower than control due to reduced placental expression of the glucose transporters (GLUT), GLUT1 and GLUT8 (Thureen *et al.* 1992, Regnault *et al.* 2003: Limesand *et al.* 2005). Conversely, the weight specific glucose transfer capacity is increased in the small placenta of carunclectomised ewes (Table 2). Similarly, 25-50% increases in placental glucose transfer capacity have been observed with prolonged maternal hypoglycaemia or restricted dietary intake when the reduced transplacental concentration gradient is taken into account (Table 2). These changes in glucose transport are accompanied by altered patterns of placental GLUT1 and GLUT3 expression, which are isoform specific and temporally distinct depending on the insult (Das *et al.* 1998; 2000; Bell *et al.* 1999; Dandrea *et al.* 2001). In contrast, when placental growth is restricted by over-nutrition of young animals, there is no change in glucose transport capacity or GLUT expression per gram placenta, despite the reductions in placental and fetal mass (Wallace *et al,* 2005). Fetal glucocorticoid over-exposure also has no effect on the weight specific capacity for placental glucose transport or on placental GLUT1 and GLUT3 expression, although umbilical glucose uptake per gram placenta is reduced as a result of increased uteroplacental glucose consumption (Table 2). The ovine feto-placental unit, therefore, adopts different strategies to help maintain a fetal glucose supply during adverse conditions depending on the specific insult. This may relate, in part, to the degree of placental growth restriction and/or to the maternal and fetal endocrine mileux.

Compared to the GLUTs, less is known about the regulation of amino acid transporters in the ovine placenta during adverse conditions. There are at least nine different amino acid transporter systems with distinct functional characteristics yet overlapping specificities that function to actively accumulate amino acids in the placenta and, then, facilitate their passive transfer into the fetal circulation (Regnault *et al.* 2005). Transplacental amino acid flux varies with the specific amino acid and the maternal concentration. It is also affected by catabolism and transamination of amino acids within the placenta itself (Regnault *et al.* 2005). In sheep, changes in the transplacental amino acid transport have been observed in response to hyperthermia, undernutrition and maternal administration of GH, IGF-I and glucocorticoids (Liechty *et al.* 1991; Liu *et al.* 1994; Ross *et al.* 1996; Harding *et al.* 1997; Timmerman *et al.* 2000). In

Table 2. Effects of environmental conditions on the characteristics of transplacental glucose transport in sheep fetuses near term (>130 days) expressed as percentage change from control values. (↑ = increase. ↓= decrease.)

Treatment	Glucose transport per kg placenta†	Transplacental glucose gradient	Uteroplacental glucose consumption per kg placenta	Glucose transport capacity per kg placenta‡	Glucose transporters	References
Nutrition						
Overnutrition	No Δ	No Δ	No Δ	No Δ	No ΔGLUT 1 & 3	Wallace et al.2002;2004
Hyperglycaemia	No Δ - ↑10%	↑ 55%	↑ 120%	?	↓GLUT1 & 3	Aldoretta et al. 1994 Das et al. 1998; 2000
Undernutrition	↓ 60%	↓ 40%	↓ 43%	↑ 50%	↑GLUT1 & 3	Leury et al. 1990 Bell et al. 1999 Dandrea et al. 1998
Hypoglycaemia	↓ 45-80%	↓ 48%	↓ 16-30%	No Δ to ↑25%	↓GLUT1 No Δ GLUT3	Aldoretta et al. 1994 Carver & Hay 1995 Das et al. 1998; 2000
Hyperthermia	↑ 25%	↑ 10%-15%	No Δ	↓ 20-30%	↓GLUT1 & 8	Thureen et al. 1992 Wallace et al. 2005 Limesand et al. 2004
Glucocorticoid treatment	↓ 20%	No Δ	↑ 70%	No Δ	No Δ GLUT1 & 3	Ward, 2002 Ward et al. 2004
Placental growth restriction††	↑ 37%	↑ 37%	No Δ	↑ 55%	?	Owens et al. 1987; 1989

† = measured as umbilical glucose uptake per kg placenta at the prevailing transplacental glucose concentration gradient. ‡ = measured either as clearance of non-metabolisable glucose analogue or as umbilical uptake per kg placenta at the normal glucose concentration gradient. †† = ewes carunclectomised before pregnancy. ? = unknown. Δ = change.

particular, there are reductions in the placental delivery of leucine, threonine, glutamate and alanine to the fetus. There are also changes in uteroplacental handling and inter-organ shuttling of essential and gluconeogenic amino acids in response to heat stress and undernutition (Liechty *et al.* 1991; Ross *et al.* 1996; Timmerman *et al.* 2000). In rodents, environmental stimuli, such as undernutrition and dietary composition, alter placental expression of the accumulative System A amino acid transporters (Jansson *et al.* 2006; Jones *et al.* 2009; Coan *et al.* 2010) but little is known about the epigenetic regulation of amino acid transporters in ovine placenta.

Placental endocrine function

The placenta produces a number of hormones including steroids, peptides, cytokines, glycoproteins and eicosanoids, which are released into both the fetal and maternal circulation (see Fowden *et al.* 2008; Fowden & Forhead 2009). Some of these hormones, such as progesterone, placental lactogen, the cytokines and placental variants of GH and prolactin have metabolic actions in the mother that favour nutrient delivery to the fetus (Gootwine 2004). In sheep, maternal concentrations of placentally derived hormones, such as progesterone and placental lactogen, are lower than normal during adverse conditions, such as hyperthermia, overfeeding, undernutrition and glucocorticoid overexposure (Regnault *et al.* 2002; Wallace *et al.* 2004; 2005; Braun *et al.* 2007). This is due, in part, to the reduced placental mass but may also reflect cyto-architectural changes in the placenta caused, for instance, by altered BNC dynamics (Table 1). Receptors for these hormones are present in the ovine placenta and binding of ligands, such as IGF-I, to their receptors has been shown to alter placental clearance of non-metabolisable glucose and amino acid analogues *in vivo* (Harding *et al.* 1994; Gootwine 2004).

Other placental hormones, such as the prostaglandins (PG)E_2 and $F_{2\alpha}$, also affect the fetal supply of nutrients and oxygen but more indirectly by actions on fetal endocrine function, regional blood flow and myometrial contractility (see Fowden & Forhead 2009). In sheep, undernutrition during late gestation increases uteroplacental activity of PGH synthase and the production of $PGF_{2\alpha}$ and PGE_2 (Whittle *et al.* 2001) These increments in PG synthesis are directly related to the degree of maternal hypoglycaemia and the fall in uteroplacental glucose consumption and can be reversed by restoring normoglycaemia by re-feeding or glucose infusion into the fasted animal (Fowden *et al.* 1994). The ovine placenta has also been shown to contain PG dehydrogenase (PGDH), an enzyme which converts biologically active PGs into their inactive keto metabolites (Whittle *et al.* 2001). Uteroplacental output of these metabolites rises towards term and in response to undernutrition during late gestation (Fowden *et al.* 1994). In part, the nutritionally induced changes in PG production and metabolism may be due to the concomitant rise in glucocorticoid concentrations as cortisol has been shown to increase production of $PGF_{2\alpha}$ and PGE_2 by enhancing PGH synthase activity and decreasing PGDH activity in ovine placenta (Whittle *et al.* 2001). Sensitivity of placental PGDH and PGH synthase to nutritional and endocrine stimuli increases with increasing gestational age in parallel with the rise in fetal cortisol concentrations and the onset of myometrial contractile activity towards term (Fowden *et al.* 1994).

The ovine placenta also inactivates a range of hormones, including glucocorticoids, catecholamines, IGFs, thyroxine (T_4) and tri-iodothyronine (T_3), which limits their effectiveness in the fetus (Fowden & Forhead 2004). For example, placental type III deiodinase converts T_4 to biologically inactive reverse-T_3 and maintains a high fetal clearance rate of T_3, the most biologically active thyroid hormone (Forhead *et al.*, 2006). Similarly, the placental enzyme, 11ß-hydroxysteroid dehydrogenase type 2 (11ßHSD2), converts active glucocorticoids to their inactive metabolites, which reduces placental and fetal exposure to the higher cortisol

concentrations found in the maternal circulation (Seckl 2004). Its placental activity is down regulated by undernutrition and both maternal and fetal administration of glucocorticoids in sheep (Whorwood *et al.* 2001; Clarke *et al.* 2002; Kerzner *et al.* 2002; McMullen *et al.* 2004; Gnanalingham *et al.* 2007). In turn, by altering placental cortisol bioavailability, these environmentally-induced changes in 11ßHSD2 activity influence placental production and metabolism of other glucocorticoid sensitive hormones, such as the PGs, progesterone, oestrogens, placental lactogen and active thyroid hormones (see Fowden & Forhead 2004; 2009). Furthermore, there are changes in the placental abundance of glucocorticoid and other hormone receptors in response to maternal undernutrition, which will affect hormone bioavailability within the placental tissues (Whorwood *et al.* 2001; Gnalingham *et al.* 2007; Yiallourides *et al.* 2009). Epigenetic changes in placental hormone production may, therefore, alter placental delivery of nutrients to the fetus either by paracrine actions on placental transport characteristics or by endocrine actions on maternal metabolism that alter nutrient allocation between the maternal and uteroplacental tissues (Fowden *et al.* 2006b). These programmed endocrine changes may also determine the length of gestation and the development of maternal tissues, such as the mammary glands, with implications for nutrition and growth after birth.

Molecular mechanisms of placental programming

Compared to the mouse placenta (Fowden *et al.* 2006a), relatively little is known about the molecular mechanisms by which environmental factors alter the morphological and functional development of the ovine placenta. Using microarrays, recent studies have shown that hypoxia and dietary protein deprivation alter expression of over 200 genes in the mouse placenta with specific up-regulation of genes involved in apoptosis and inhibition of cell growth (Gheorghe *et al.* 2010). Gene deletion studies have demonstrated that imprinted genes, which are expressed monoallelically in a parent-of-origin manner, have a disproportionately important role in placental development (Reik *et al.* 2003; Fowden *et al.* 2006b). In particular, the imprinted *Igf2-H19* gene locus has been shown to affect both trophoblast morphology and nutrient transfer (Reik *et al.* 2003; Coan *et al.* 2008b). Deletion of the placental specific P0 transcript of the *Igf2* gene causes placental growth retardation but increases placental efficiency in association with up-regulation of placental glucose and amino acid transfer and of placental expression of *Slc2a3*/GLUT3 and *Slc38a4*, an isoform of the System A amino acid transporters (Constancia *et al.* 2002; 2005). During late gestation, expression of this *Igf2P0* transcript is altered in conjunction with changes in nutrient transfer by maternal dietary manipulations and when placental growth is restricted naturally (Coan *et al.* 2008a&b; 2010). Collectively, these studies suggest that placental *Igf2* has a major role as an environmental sensor and adapts placental phenotype to help support fetal growth in mice.

Both IGF-II and IGF-I are expressed in ruminant placenta, particularly early in gestation, as is the primary IGF receptor, IGF1R (Wooding & Burton 2008). In ovine placentomes, expression of the IGFs, IGF1R and their binding proteins is altered in a regional and temporally specific manner by environmental factors including hyperthermia, maternal GH administration, overnutrition and both acute and chronic under-nutrition at various stages of pregnancy (Osgerby *et al.* 2004; McMullen *et al.* 2005; de Vrijer *et al.* 2006; Gnanalingham *et al.* 2007; Wright *et al.* 2008; Yiallourides *et al.* 2009). Indeed, the smaller the placenta the higher the IGF-II abundance in moderately nourished ewes (Osgerby *et al.* 2003), which suggests that placental IGF-II may act to enhance growth of the compromised placenta, as occurs in mice (Coan *et al.* 2008b). In addition, both under and over-nutrition alter the intracellular signalling pathways downstream of IGF1R in ovine placentas (Zhu *et al.* 2007; 2009), which has implications for

placental protein synthesis, cell proliferation and expression of nutrient transporters, independently of IGF concentrations (Jansson & Powell 2007).

At the chromatin level, epigenetic regulation of gene expression can occur by changes in DNA methylation, histone modifications and/or siRNA abundance (Gluckman *et al.* 2009). In somatic tissues, changes in DNA methylation and histone modifications have been observed in the promoters of key metabolic genes in the adult offspring of rats malnourished or glucocorticoid treated during pregnancy, which parallel alterations in gene expression and enzyme activity (see Burdge *et al.* 2007). Changes in hepatic DNA methylation have also been observed in rat and sheep fetuses of mothers fed methyl deficient diets (Rees *et al.* 2000; Sinclair *et al.* 2007). In part, this may be due to reduced hepatic activity of DNA methytranferase responsible for maintaining DNA methylation during cell replication (Lillycrop *et al.* 2007). Even less is known about these processes in the placenta. When histone H3 trimethylation is prevented in mice by deletion of a specific histone methytransferase, vascular development of the definitive hemochorial placenta is impaired as embryonic blood vessels fail to invade the labyrinthine layer, resulting in embryonic lethality at 11.5 days (Hu *et al.* 2010). Similarly, inhibition of DNA methylation by 5-azacytidine administration to pregnant rats, leads to a small placenta at term with impaired development of the labyrinthine trophoblast (Šerman *et al.* 2007). Hypomethylation and biallelic expression of the *H19* gene locus is also seen in the overgrown placenta of cloned cattle (Curchoe *et al.* 2009). In addition, hypomethylation of the imprinting control region of the *IGF2/H19* domain, or of the domain itself, has been observed in placentas of growth restricted human infants in some but not all studies (Tabano *et al.* 2010; Bourque *et al,* 2010). Genes involved in DNA methylation and histone modifications are down-regulated in mouse placenta after maternal hypoxia and protein deprivation during the second half of pregnancy but whether this alters the methylation status of any of the other affected placental genes remains unknown (Gheorghe *et al.* 2010). Maternal undernutrition was not associated with altered methylation of the promoter regions of the *Igf2* or *Slc38a4* genes in the mouse placenta, despite changes in their expression in late gestation (Coan *et al.* 2010).

Conclusions

Environmental factors have an important role in programming the placental phenotype (Figure 2). They may act, directly, on placental development or, indirectly, through changes in the maternal endocrine environment (Figure 2). Together, they programme the nutrient transfer capacity of the placenta by altering its size and morphology, its transport characteristics and its endocrine function. In turn, these placental adaptations affect the fetal endocrine environment and the absolute and relative quantities of nutrients supplied to the fetus with consequences for intrauterine development and offspring phenotype (Figure 2). They also alter maternal adaptation to pregnancy and the allocation of maternal resources to feto-placental development. The memory of early environmental events can, therefore, be transmitted to the fetus long after the original insult through the placental epigenome. Thus, the placenta is not just a passive conduit for nutrients but adapts its nutrient transfer capacity dynamically during environmental challenges to optimise fetal acquisition of nutrients for growth given the prevailing nutrient availability.

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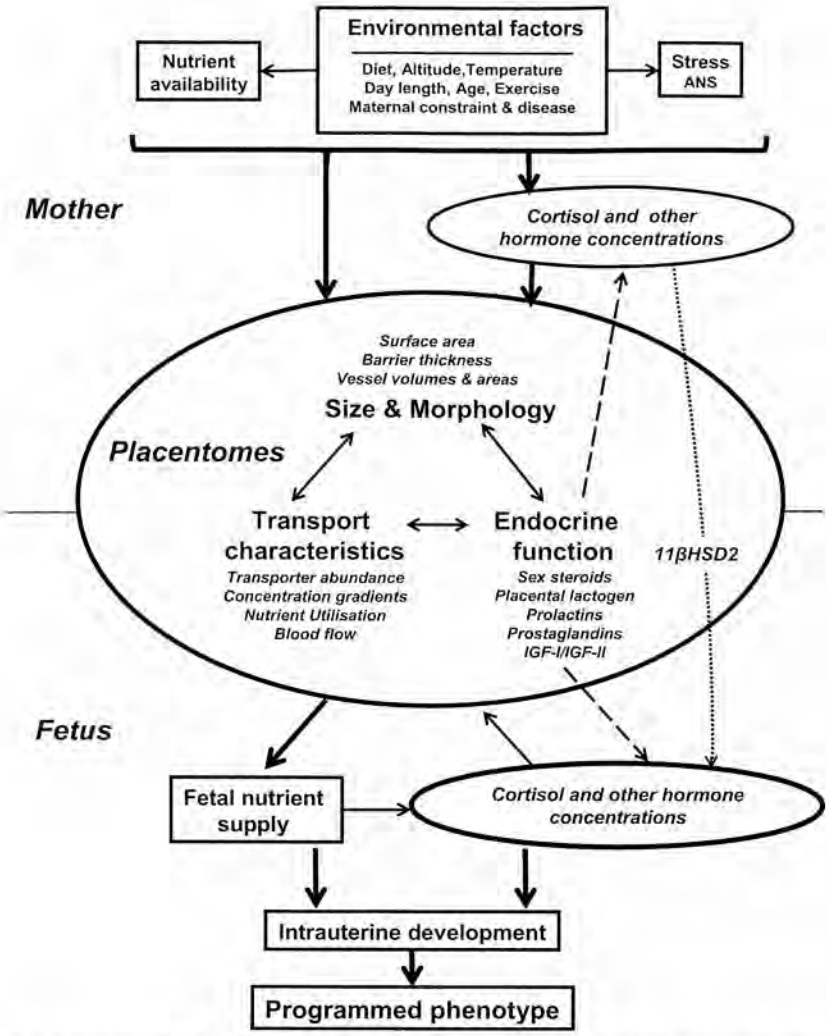

Fig. 2. Schematic diagram showing the role of environmental factors in controlling placental phenotype and the consequences of these regulatory actions for intrauterine development and developmental programming of the offspring. ANS, Autonomic Nervous System. 11ßHSD2, 11ß-hydroxysteroid dehydrogenase type 2. IGFI/IGFII, Insulin-like growth factors I and II.

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