Intrauterine fetal growth restriction, adaptation and programming–a review

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Abstract

Intrauterine fetal growth restriction (IUGR) is fetal adaptation in response to a wide range of pathophysiological factors, including an inadequate supply of oxygen, nutrients, or both. In particular, low birth weight has been associated with subsequent disorders in adult life, including cardiovascular, neuro-developmental, metabolic, and reproductive diseases, independent of adult life style. Animal models are excellent tools to study the effects of genetic, molecular and cellular events that modulate intrauterine growth and development. Various experimental approaches have been used, involving alteration of numerous metabolic and physiologic events, with substantial variations among studies in both methods and species used. Intrauterine fetal growth restriction remains a challenging problem in humans and is a major concern in agricultural animal since it affects production parameters. The objective of this manuscript is to review animal models which explore fetal adaptation, including IUGR, and evaluate their outcomes, to suggest new opportunities for further research, and the potential for interventions to reduce or eliminate gestational events with deleterious effects on fetal, neonatal, and adult health.

Keywords: Fetus, placenta; animal model; growth restriction

Introduction

Intrauterine fetal growth restriction results in unduly high perinatal morbidity and mortality.[1-4] Intrauterine fetal growth restriction refers to a condition in which a fetus is unable to achieve its genetically determined potential size. The clinical description of IUGR is on the basis of birth weight below the tenth percentile, or fetal body weight less than two standard deviations from the mean of study population.[5,6] Several pathophysiological factors can disturb or restrict fetal growth, including chromosomal abnormalities, gene defects, poor placental function, and maternal stress due to environment or nutrition. The physiological adaptations of the fetus to an adverse intrauterine environment in response to pathophysiological factors, including restrictions on its growth rate, depend on the nature, timing and intensity of extrauterine or intrauterine challenges. Adaptation of the fetus to a suboptimal intrauterine environment is of clinical importance in determining long-term, postnatal health.[7] The physiological, neuroendocrine or metabolic adaptations that help the fetus to survive a period of intrauterine deprivation result in permanent programming of proliferation and differentiation events within key tissue and organ systems and pathological consequences in adult life.[8,9] This is the so called Barker's hypothesis, after David J. P. Barker, who published the theory in 1997.[7] The magnitude of fetal adaptations for both in utero survival and postnatal health outcomes provided the momentum for experimental studies to elucidate the nature and consequences of specific fetal adaptations to a compromised or poor intrauterine environment. This review summarizes animal model studies focused on the range of adaptations the fetus makes in response to a suboptimal uterine environment.

Underlying mechanisms

Normal growth has been described as, "the idiom of the genetic potential to grow which is neither abnormally constrained nor promoted by internal or external features". In this definition, internal features relate to the fetus, whereas external features relate to the placenta, uterus, dam, and environment. However these internal and external characteristics, including the fetus, dam, and utero-placental unit, act collectively; therefore, the problem of interactions among the fetus, placenta and dam must be considered. Furthermore, the genetically determined growth course at each stage of gestation has to be understood before growth restriction can be imposed. However, due to inherent interactions among numerous components, it is very difficult to determine the physiologic growth curve of an individual fetus.[10] In

addition a fetus can achieve normal fetal growth despite altered internal and/or external features, since the maternal, utero-placental and fetal tissues are capable of adapting to alterations. Furthermore, since the placenta and fetus are developing, they are not in a steady state. Consequently, the same experimental method may have different effects, depending on the stage of gestation when it is applied, emphasizing the need to carefully consider the timing of experimental alterations.

Normal intrauterine growth takes place in phases–an embryonic and a fetal phase. The embryonic phase consists of proliferation, organization and differentiation of the embryo, whereas the fetal phase consists of continuing growth and functional maturation of the various tissues and organs. The fetal phase of intrauterine development depends on genetic, placental, and maternal factors. According to time of adverse intrauterine nutrition, a fetal growth restriction is classified as symmetric or asymmetric,[11] as shown (Table 1).

Table 1: Comparison of symmetric and asymmetric fetal growth

Symmetric	Asymmetric
Occurs early in gestation	Occurs late in gestation
Entire fetus is proportionately small	Fetus is normal length but reduced weight
Multifactorial - genetic, infectious or toxic effects	Due to placental insufficiency
Postnatal make-up growth is rare	Postnatal make-up growth possible

Adapted from 11

Factors that cause fetal growth restriction

Numerous factors can result in restriction of fetal growth, including:

Maternal factors

- Undernutrition
- Maternal low birth weight
- Maternal age
- Chronic hypertension (gestational induced)
- Pre-eclampsia
- Diabetes

Environmental factors

- Toxicity
- High altitude
- Hyperthermia
- Nutritional deficiencies

Placental factors

- Abnormal placentation
- Chronic abruption, infarcts, focal lesions
- Ischemia
- Chronic inflammatory conditions (e.g. villitis or placentitis)

Fetal factors

- Chromosomal anomalies
- Genetic conditions
- Congenital malformations
- Intrauterine infections
- Multiple fetuses

Impacts of fetal programming on postnatal life

- Neurologic, endocrine and cardiac problems
- Diabetes
- Delayed puberty

- Poor ovarian follicular development
- Fewer germ, Sertoli and Leydig cells
- Poor placentation/fewer uterine carunlces
- Poor uterine capacity
- Reduced litter size
- Poor postnatal growth
- Poor meat quality

Treatment modalities

- Exogenous progesterone or synthetic progestins
- Exogenous growth hormone
- Dietary supplementation of energy, protein, or both
- Antioxidants
- Manipulations of arginine-nitric oxide/polyamine
- Sildenafil citrate
- Aspirin

Experimental models of restriction of intrauterine fetal growth

Various experimental approaches have been used to induce IUGR in several species, including mice, rats, guinea pig and sheep (it occurs spontaneously in virtually all species). Experimental methods to induce IUGR include a reduction in utero-placental blood flow by vascular occlusion or ligation, a reduction in materno-fetal contact by cornual ligation or removal, placental embolization, caruncleclectomy, genetic manipulation of the fetus, fetal infection, a reduction in maternal nutrient intake (energy, protein, or both), maternal overfeeding, and maternal hyperthermia (Figure 1).[12-40]

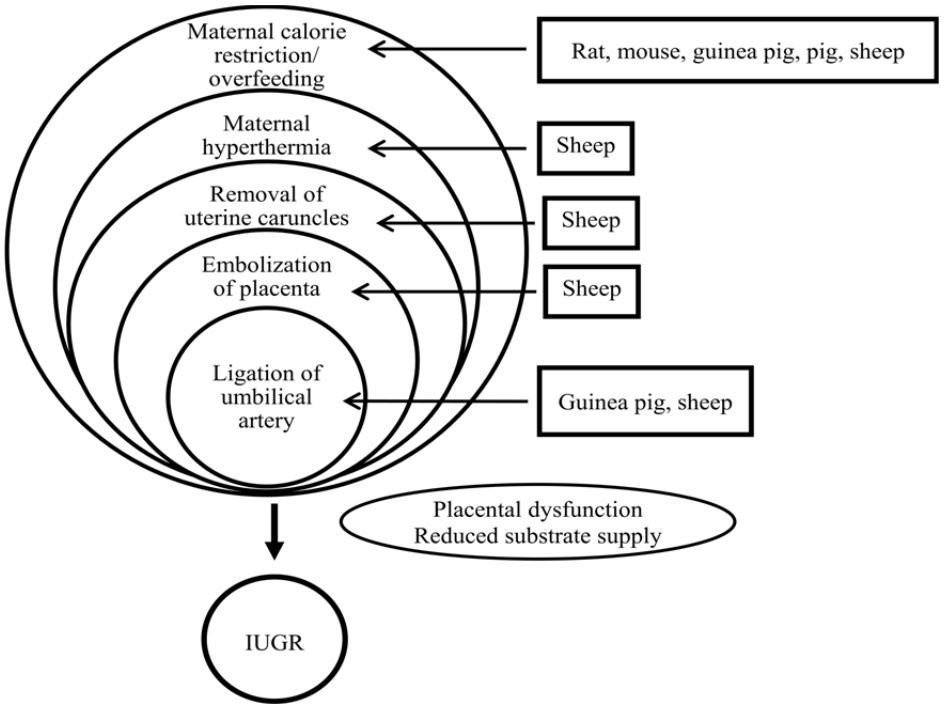

Figure 1: Experimental approaches to produce intrauterine fetal growth restriction (IUGR) in animals.

Both nutritional and placental insufficiency models resulted in IUGR and postnatal maladies, including endothelial dysfunction, hypertension, insulin resistance, type 2 diabetes, reduced cognitive

development, and cardiac diseases. Fetal growth parameters and hormones in the ovine IUGR fetus relative to a control are shown (Table 2); that the IUGR fetus weighed less than the control validated the model.

Table 2: Fetal growth parameters and hormones in the growth-restricted ovine fetus (relative to control)

	Carunclectomy	Hyperthermia	Maternal overfeeding	Embolization	Uterine artery ligation
Birth weight	↓	NA	↓	↓	NA
Placental weight	↓	↓	↓	↓	↓
Fetal weight	↓	↓	↓	↓	↓
Relative organ weight	↑/=	↑/=	↑/=	↑/=	↑/=
Fetal plasma ACTH	↑			↑	
Fetal plasma cortisol	↑	= females; ↑ males	=	↑/=	↑
Noradrenaline	↑	↑		↑	

Adapted from 40-42

Placental vascular development

The placenta has a major role in regulation of fetal growth. The sow and mare have a diffuse placenta, with chorionic villi distributed over the entire surface of the chorion, whereas in ruminants the chrioallantois attaches to discrete sites on the uterine wall. Placental area of attachment continues to increase as gestation advances[43,44] and vascular development of the placenta increases ~200% from mid- to late gestation.[44] In the cow, cotyledonary growth progressively increases throughout gestation,[45,46] whereas in the ewe, growth of the cotyledonary mass is exponential during the first 70–80 days of pregnancy, thereafter slowing markedly until lambing.

Table 3: Comparison of percentage change in capillary vascularity from mid- to late pregnancy in sheep (Days 50 to 140) and cattle (Days 125 to 250)

Placentomal tissue	Vascularity parameter	Sheep	Cattle
Caruncle	CAD	↑ 214%	↓30%
	CND	↑ 37%	↑151%
	CSD	↑140%	↑32%
	Capillary size	↑45%	↓68%
Cotyledon	CAD	↑437	↑186%
	CND	↑1093%	↑80%
	CSD	↑576%	↑172%
	Capillary size	↓ 25%	↑71%

CAD - Capillary area density (a flow-related measure); CND - capillary number density (an angiogenesis-related measure); CSD - capillary surface density (a nutrient exchange-related measure).

Studies on changes in placental vascularity in response to nutrient-restriction in ewes and cows are very limited, and appear to be largely lacking in sows. Seven days of fasting during mid-pregnancy in the ewe decreased vascular endothelial growth factor (VEGF) mRNA levels and placental weights on Day 90; however, placental weights were similar at lambing in nutrient-restricted and control ewes.[47] In nutrient-restricted cows (Days 30 to 125 of gestation) there was a decrease in total placentome weight, including both the cotyledonary and caruncular portions, on Day 125 compared to control cows. This inhibition in total placentome weight persisted, even after realimentation until Day 250;[46,48] however, only the weight of the cotyledonary tissue remained suppressed at Day 250.[48] In contrast, in several sheep models of maternal nutrient restriction from early to mid-pregnancy followed by realimentation, there was significant compensatory growth of the entire placentome.[46,49] Although maternal nutrient delivery

during pregnancy has been shown to program fetal growth and development, both during pregnancy and later into adult life, it appears that maternal nutrition also programs placental development. In the cow, realimentation after ~90 days of nutrient restriction is the stimulus not only for altering placental vascularity and development, but also placental function.[50,51]

Animal models

Several researchers used animals to study various aspects of fetal development. Those research scenarios could be used as animal models and compared with existing fetal growth restriction models to advance knowledge regarding mechanisms underlying IUGR. Some scenarios worthy of investigation include:

1. Large offspring syndrome in cows
2. Uteroplacental hypoxia in sheep
3. Embryonic/fetal adaptation in mares
4. Low birth weight piglets

Nutritional status and environmental events can alter important physiological parameters of the embryo and fetus. This resetting is called as fetal or developmental programming and it can continue into childhood and adulthood to trigger disorders. The fetal cellular environment can modify the gene expression pattern in various tissues and organs, with long-term functional consequences in neonatal period and adulthood. Fetal programming involves primary alterations and secondary compensations. According to the concept of fetal programming, any detrimental circumstance during the critical period of in-utero development affects the subsequent pattern of growth and development of tissues and organs and may predispose the affected fetus to metabolic disorders later in life.[7]

The placental angio-architecture is vulnerable to any alterations in the maternal environment. Adverse uterine milieu produces an altered placental vascular phenotype and function. In utero, adverse events are critical factors in determining fetal growth, quality of life and overall health. Underlying mechanisms of fetal programming caused by various perturbations in the maternal compartment and how they are transmitted across the placenta remain to be completely established. Several vascular factors including bFGF, EGF, PDGF, angiopoietin 1 and 2, and the VEGF family, have been identified as important placental angiogenic regulators. Up-regulation of angiogenic factors VEGF, bFGF and eNOS have been documented in IUGR pregnancies. Differential expression of pro- and anti-angiogenic factors such as vascular endothelial growth factors (VEGF) and placental growth factors (PlGF) throughout gestation indicates that both play essential roles in placental angiogenesis.[52-54] The imbalance of pro- and anti-angiogenic mediators during gestation results in impeded fetal growth and other complications.

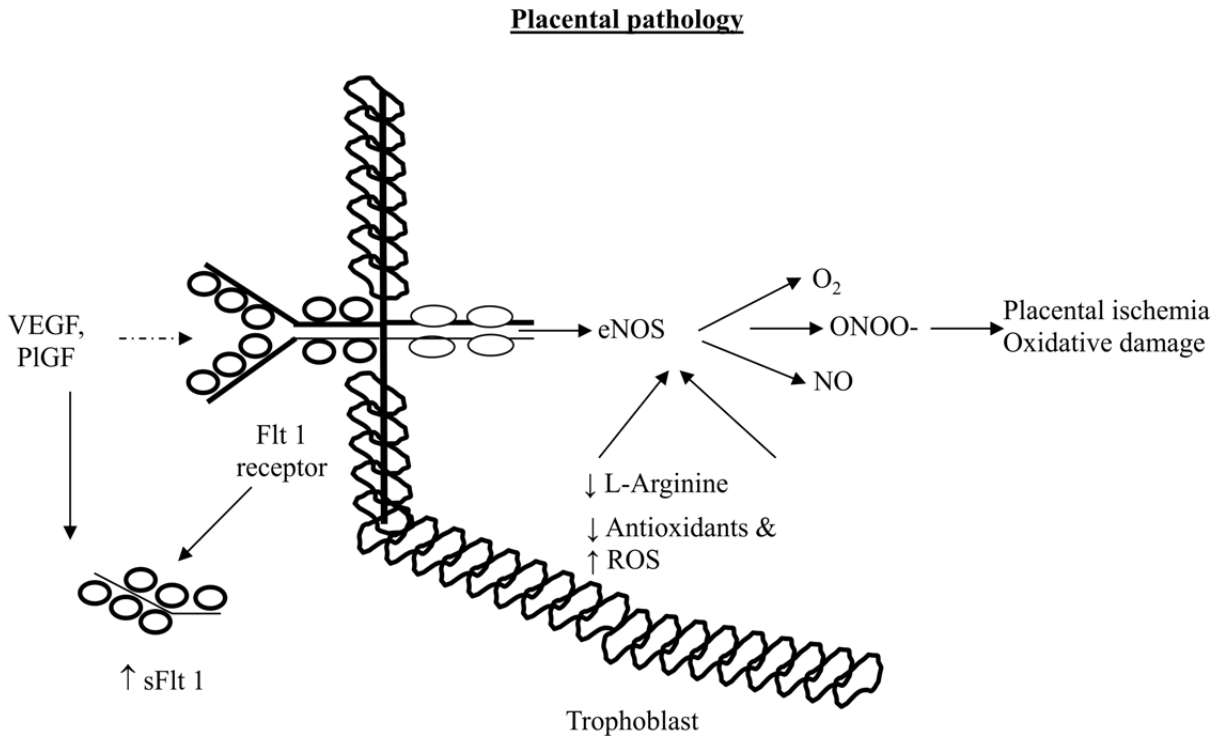

Figure 2: Imbalance of pro- and anti-angiogenic mediators during gestation results in oxidative damage, placental ischemia, impeded fetal growth, and other complications.

Large offspring syndrome in cattle

In vitro production of bovine embryos often affects embryonic gene expression and organogenesis, leading to large offspring syndrome (LOS). In cattle, the developmental competence of in vitro produced embryos is compromised compared to in vivo embryos. Embryos produced in vitro differ from those produced in vivo with regards to morphology, timing of development, embryonic metabolism, intercellular communication, cell number, and gene expression.[55-61] A recent study compared three embryo production systems to identify genes affected by in vitro processes and associated with the LOS; 1) embryos produced in vitro (IVF), 2) embryos fertilized in vivo followed by in vitro culture (in vivo derived, IVD), and 3) embryos generated entirely in vivo by artificial insemination (AI-IVD).[47] There were 306, 367, and 200 genes differentially expressed between the AI-IVD, IVF and IVD, and AI and IVF comparisons, respectively. Interestingly, 44 differentially expressed genes were identified between the AI embryos and both the in vitro and in vivo embryos, making these genes potential candidates for LOS. Comparing these genes in an IUGR model would provide additional insight into fetal programming. In another study, in vitro produced bovine embryos cultured in high concentrations of either serum or bovine serum albumin (BSA) had significantly more cells in Day 7 blastocysts, a larger blastocyst on Day 12, and differences in relative abundance of transcripts for heat shock protein 70.1, Cu/Zn-superoxide dismutase (SOD), glucose transporters-3 (Glut-3), Glut-4, bovine-fibroblast growth factor (bFGF), and insulin-like growth factor 1- receptor (IGF1-R) when compared with in vivo embryos; these differences provided evidence that the culture system had an important role in development.[61] Furthermore, calves derived from in vitro embryos were significantly heavier than those derived from sheep oviduct culture, superovulation, or AI.[62]

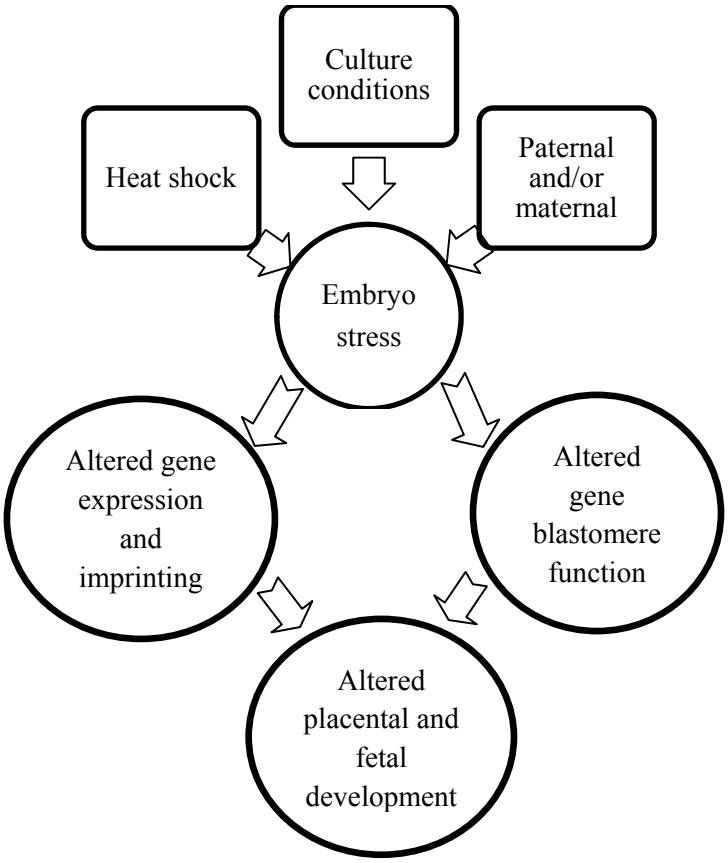

Figure 3: Factors altering fetal and placental development and fetal programming (adapted from 47-49)

Cloning by nuclear transfer of somatic cells (SCNT) is associated with high rates of embryonic and fetal mortality in various species.[63,64] In bovine pregnancies with a fetus produced by SCNT, a poorly developed placenta with very few placentomes and limited vascularization has frequently been described during early pregnancy;[63,64] that the extra-embryonic tissues fail to develop normally in SCNT clones causes abnormalities in implantation and placental development. In placentas of SCNT pregnancies examined between 180 and 280 days of gestation, there were indications that placental overgrowth preceded fetal overgrowth,[64,65] perhaps due to alterations in placental genes during early development. For the fetus to survive, the placenta must overcome and compensate for developmental defects caused by programming errors; furthermore, in cloned offspring, adaptation to these changes may cause IUGR.[65] Altered placental development due to SCNT-induced genomic perturbations would be consistent with such observations.

It is apparent that the pre-implantation embryo is extremely sensitive to subtle changes in environment with long-term consequences for the health of the offspring. Genes that are implicated in these studies can be studied further using gene silencing or transfection models to determine their pathophysiology in the development of placentation and IUGR.

Uteroplacental hypoxia in sheep

Intrauterine growth restriction, often associated with functional placental insufficiency, increased perinatal mortality and morbidity. Although no animal model can fully simulate human pregnancy, the pregnant sheep has been used extensively to investigate maternal-fetal interactions.

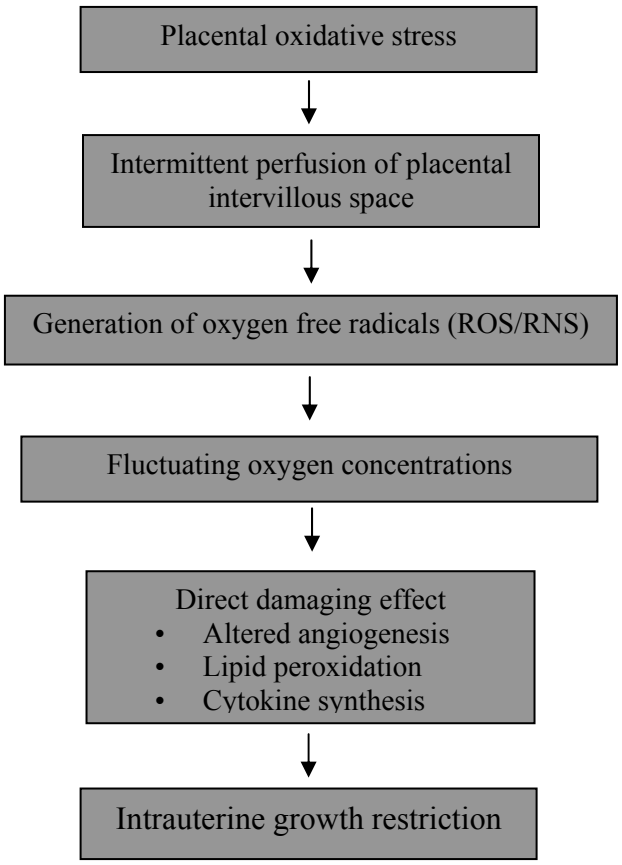

Figure 4: Placental oxidative stress and intrauterine growth restriction. Placental oxidative stress induced by generation of an abundance of oxidative free radicals cause lipid peroxidation and alters angiogenesis, which reduces nutrient delivery to the fetus, causing fetal growth restriction.

Development of the placental vascular tree is regulated by several growth factor families. Based on differential expression of vascular endothelial growth factors (VEGF) and placental growth factors (PlGF) throughout gestation, both have essential roles in placental angiogenesis.[66-68] The latter is divided into two phases, branching and non-branching.[66-68] Branching angiogenesis, which occurs during the early phases of gestation under relatively hypoxic conditions, is responsible for expansion of the pre-existing vascular bed, and is driven by VEGF. Non-branching angiogenesis promotes elongation of pre-existing capillary loops and occurs later in gestation under the influence of PlGF. The balance of these two growth factors throughout gestation is responsible for formation of a normal placenta.[67,68] The increasing transplacental oxygen gradient generates changes in angiogenic growth factors which may be associated with the underlying pathophysiology of post-placental hypoxic fetal growth restriction. An altered state of placental oxygenation is associated with aberrant villous morphology.[69-71] A fetal growth restricted placenta that develops under maternal hypoxia (e.g. at a high altitude) is characterized by increased branching angiogenesis and reduced vascular impedance.[72,73] In contrast, pregnancies with restricted fetal growth are characterized by the absence of end diastolic flow in the umbilical artery and increased vascular impedance, predominantly associated with non-branching angiogenesis.

Placental oxidative stress induced by ischemic perfusion has been implicated in the pathophysiology of fetal growth restriction. Placental tissues from IUGR pregnancies have decreased placental anti-oxidant capacity, and lower mRNA expression of Cu/Zn superoxide dismutase (an important enzymatic antioxidant) and non-enzymatic antioxidant vitamin E.[74-76] Based on several studies, decreased bioavailability of endothelium-derived NO, due to oxidative destruction of NO by ROS, may

contribute to the impaired endothelium-dependent vasodilatory responses, for which treatment with antioxidant vitamins apparently have a beneficial role.[74-76]

Tocopherols are micronutrients which act as free radical scavengers, i.e. chain-breaking antioxidants. The major tocopherols in mammalian tissue are alpha and gamma. Under specific circumstances, gamma tocopherol might scavenge reactive species more effectively than alpha tocopherol.[77] In that regard, gamma tocopherol was 35% more effective than alpha tocopherol at inhibiting systemic lipid peroxidation. Furthermore, gamma tocopherol is incorporated into the endothelial cells more rapidly than alpha tocopherol, and supplementation with gamma tocopherol was significantly more effective than alpha tocopherol at inhibiting iron-induced lipid peroxidation and occlusive thrombus formation.[78] In addition to free radical scavenging properties, since gamma tocopherol has specific activities with key inflammatory enzymes, it may have an added advantage of suppressing inflammation during pregnancy. Supplementation of tocopherols may reduce utero-placental hypoxia and promote placental vascular development by reducing hypoxia.

The sheep may be an appropriate model to study methods to overcome placental hypoxia, namely, the use of antioxidants to promote vasculogenesis.

Embryonic/fetal adaptation in mares

At the histological level, the exterior surface of the allantochorion in mares consists of highly complex microplacentomes, each packed with fetal and maternal capillaries, which enables materno-fetal exchange of nutritional, gaseous and waste products.[79-84] A healthy and functional feto-maternal unit (placenta and uterus) is prerequisite for a successful pregnancy in the mare;[84] anything affecting this fetomaternal attachment may result in abortion or weakness in the newborn foal. In that regard, intrauterine development of the fetus and fetal birth weight are closely related to the total area of contact of the allantochorion.

In mares, placental compromise is evident in twinning, age-related endometrial degeneration, and maternal size. In twinning, allantochorions of the two conceptuses compete for the limited surface area of endometrium, whereas in endometrial degeneration, the area of placental exchange is reduced. Placental compromise related to maternal size has been well characterized by crossing large Shire horses with small Shetland ponies; the foal from the Shetland mare weighed only half that of its half sibling born from the Shire mare. Importantly, this difference persisted into adult life. Tischner created three pairs of sex-matched, full sibling Konik pony foals, whereby one of each pair had been transferred as a blastocyst to the uterus of a larger draft-type recipient mare, whereas the sibling was carried to term in its genetic pony mother.[85] All three transferred foals were bigger at birth and grew faster while nursing their larger surrogate mothers.[86] In other studies, embryos were transferred between thoroughbred (T) mares and pony (P) mares to establish four types of pregnancies: T-in-P, P-in-T, and, as controls, T-in-T and P-in-P. The T-in-T foals were heaviest and the P-in-P foals were lightest at birth.[87] The P-in-T foals were heavier than their T-in-P counterparts. Further, differences in birth weight were paralleled by differences in placental mean weight, volume, total surface area and density of microcotyledons on the allantochorion and the total microscopic area of fetomaternal contact at the placental interface. Thus, intrauterine development of the fetus and the resulting size of the foal are closely related to the total area of contact of the allantochorion.

The principal vasculogenic and angiogenic factor VEGF, and its two major receptor molecules, Flt and KDR, are localized throughout most of gestation on the two principal secretory cell types of the equine placenta, the glandular and lumenal epithelia of the maternal endometrium and the trophoblast of the fetal allantochorion.[88] Perhaps, VEGF, Flt and KDR interact in the mare to facilitate development of maternal and fetal vascular networks for exchange of gases, nutrients and waste products throughout gestation. In this regard, mares could be used as model to study fetal adaptation during development. The use of T-P models will address both the restricted (T-P) and over-fed (P-T) scenarios. Questions addressed by these studies include:

1. What are differences in the placental vasculogenesis gene expression in restricted and over-weight maternal conditions?

2. What are differences in gene expression when there is fetal growth restriction in contrast to fetal adaptation in those conditions?

Low birth weight in piglets

In pigs, birth weight is a major determinant of postnatal survival. Since attempts to alleviate the detrimental effects of low birth weight after birth have achieved only limited success, it appears that the in utero environment during a critical stage of gestation can program fetal gene expression and metabolism, leading to low birth weight. The capacity of the placenta to transport nutrients has a direct effect on fetal growth. The placenta supplying the smallest fetus in the uterus is disproportionally lighter than those supplying average-sized fetuses in the same litter.[89] Furthermore, there is a positive relationship between placental blood flow and fetal weight.[90] Therefore, there is a generalized reduction in the ability of placentas supplying small fetuses to deliver nutrients.

Supplementation of L-Arginine has been studied extensively studied using pig models. Arginine, a conditionally essential amino acid,[91] is the nitrogenous precursor of nitric oxide (NO), a vasodilator and a cell signaling molecule.[92,93] It has been established that NO and other products of arginine catabolism, e.g. proline and polyamines, are crucial for cell growth, migration, and proliferation, as well as angiogenesis.[94] Gilts were supplemented with 0.0, 0.4, or 0.8% L-arginine between 0 and 25 d of gestation.[95] Dietary supplementation with 0.4 or 0.8% L-arginine enhanced its concentrations in maternal plasma (64 and 98%, respectively) as well as the vascularity of chorionic and allantoic membranes, compared to the control group.[95] Reproductive performance, including numbers of corpora lutea and fetuses, as well as placental and fetal weights, and embryonic mortality, did not differ between the 0.4% L-arginine and control groups. However, supplementation with 0.8% L-arginine between 0 and 25 d of gestation, increased placental vascularity, but adversely affected reproductive performance of gilts.[92] Interestingly, giving rats 1.2% L-arginine for 7 d (immediately after breeding) reduced embryonic mortality by 30% .[96] Similarly, supplementing 0.83% L-arginine to gilts between 30 and 114 d of gestation increased the number of live-born piglets by two per litter.[97]

The pig may be an appropriate model for experiments dealing with both birth weight and neonatal nutrition. There can be up to a 3-fold difference in body weight among littermates in normally fed sows, thus providing a natural form of intrauterine growth restriction.[98,99] In addition, this model allows artificial rearing, which facilitates modulation and control of feed intake during the neonatal period. Moreover, comparisons of selection methods based on uterine capacity over treatment modalities could also be evaluated.

Fetal programming

Epigenetic refers to the study of changes in gene function that may be mitotically and/or meiotically inherited, but are not related to changes in DNA sequence.[100] Several mechanisms of epigenetics have been reported, including DNA methylation, modification of histones and modification of gene expression by non-coding small interfering or long RNAs.[101] Interestingly, miRNAs down-regulate or weaken several target genes; and the key mechanisms underlying this process are DNA methylation and histone acetylation.[100] These molecular mechanisms effectively determine if gene transcription or gene silencing occurs. In brief, histone acetylation and deacetylation are associated with gene transcription and silencing respectively. The histone tails can also be methylated and can activate or silence gene expression. Other examples of epigenetic modifications include phosphorylation, ubiquitinization and sumoylation.[102] Epigenetic mechanisms are important in imprinting where one gene allele derived from one parent is silenced, whereas the allele of the same gene but inherited from the other parent is expressed.

Embryonic development is characterised by two periods of epigenetic programming, which occur during gametogenesis and the pre-implantation period of pregnancy, respectively. The pre-implantation embryo is extremely sensitive to subtle changes in the environment, with long-term consequences for the health of the offspring. Although underlying mechanisms of epigenetic perturbations are unknown, there are indications of altered epigenetic programming of embryo and endometrium, particularly at the

maternal-fetal interface. Suboptimal physiology of the endometrium can subtly or substantially affect embryo development before implantation, with visible and sometimes severe consequences on placentation, fetal development and pregnancy outcome. Thus, whereas pregnancy outcome relies on embryo quality, it appears that the developmental course of the embryo is also epigenetically driven by endometrial mechanisms from the very earliest stages of pregnancy.[103] In the context of assisted reproductive technologies, developing diagnostic and prognostic tools based on endometrial factors will be valuable, with objectives to estimate the reproductive capacity of the mother, to correct suboptimal endometrial functionality and to assess the developmental potential of the embryo. On a more fundamental basis, an essential issue will be to carefully analyse situations in which embryos can overcome the control exerted by the endometrium.[104] In ruminants, a major difficulty in elucidating pregnancy-related gene functions at the materno-fetal interface is the number of genes to be analyzed. To bypass this problem, alternative options have been published. In that regard, lentiviral infection of blastocyst-stage embryos is an effective approach to study trophoblast gene function.[105-107]

As mentioned above, fetal programming may be achieved through several mechanisms. At a cellular level, programming affects aspects of cell cycle dynamics and apoptosis, whereas at the subcellular level, telomerase shortening and altered function of various enzyme systems have been documented. These are important mechanisms during development and differentiation, allowing various tissues and organs to develop from the same original genotype. The consequence of methylation in nutritional programming has been ably demonstrated in animal models. A sheep model with methionine deficiency (induced by dietary deficiency of vitamin B12 and folate; Figure 5) around the time of conception, demonstrated that male offspring were adipose and insulin resistant, and that these changes were associated with methylation of several genes.[108,109] These experiments highlighted an important role in nutritional programming. Perhaps reductions in dietary methionine and folate during the periconceptional period will cause widespread epigenetic alterations to DNA methylation in offspring, and modify adult health-related phenotypes.[110] Furthermore, genes which increase susceptibility to diabetes also reduce birth weight by influencing insulin-mediated growth, i.e. low birth weight and type 2 diabetes are both phenotypes of the same genotype.[109,111] It was observed that the rare mutation in glucokinase gene (GCK) reduces insulin secretion in β-cells. If the GCK mutation was inherited paternally, birth weight was reduced by 500 gm, provided the mother was normal. However, if the baby inherited the GCK mutation maternally, birthweight was normal (since maternal diabetes negated the effect of mutation) or it was 500 g above average if the mutation was not inherited.[112] Maternal under-nutrition and lower socio-economic status remain the most important causes of low birth weight worldwide. Numerous animal models of under-nutrition and micronutrient supplementation have confirmed the role of nutritional fetal programming.

Imprinted genes have diverse functions, notably including regulation of fetal growth.[113,114] Much attention has been directed to the IGF signaling pathway, which has a major influence on fetal size. This pathway contains two components encoded by oppositely imprinted genes, namely, IGF2 (a growth promoting factor from the paternal allele) and IGF2R (a growth inhibitory factor from the maternal allele). These genes fit the parent-offspring conflict hypothesis for the evolution of genomic imprinting.[115,116] It appears that not all components of growth regulatory pathways are encoded by imprinted genes; perhaps within a pathway, the influence of a single gene by each of the parental genomes may be sufficient for the parent-offspring conflict to be enacted. Several imprinted genes are known to influence energy homeostasis. In that regard, some, including IGF2 and growth factor receptor-bound protein 10 (GRB10), may coordinate growth via glucose-regulated metabolism.[117-119]

Since perturbation of fetal growth can be associated with metabolic disorders in adulthood, these imprinted genes are considered candidates for involvement in this phenomenon of fetal programming.

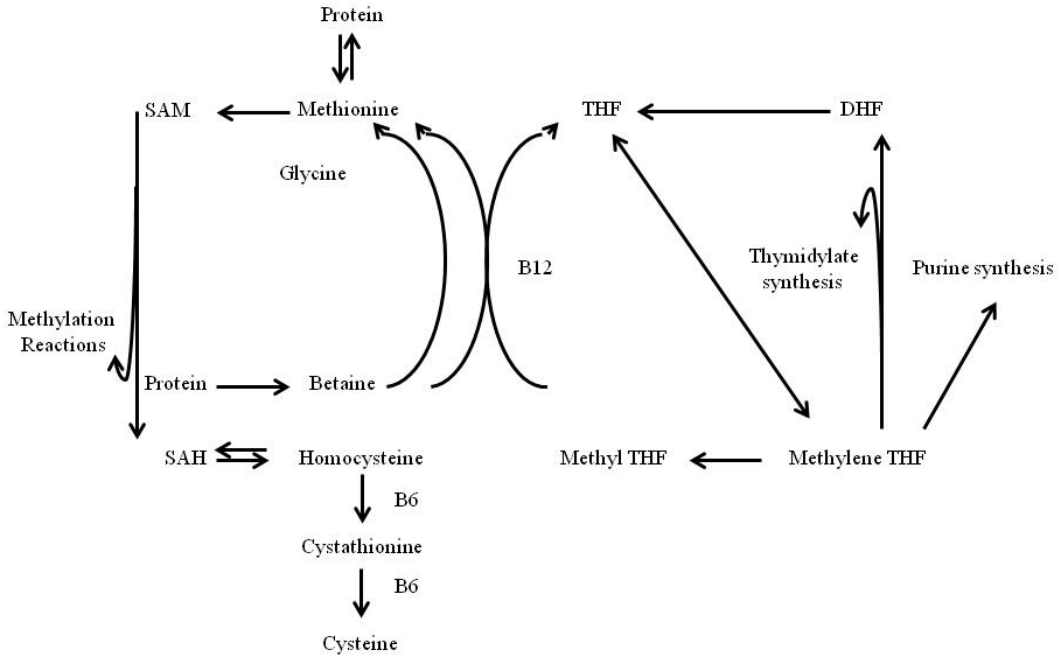

Figure 5: Nutrition and DNA methylation. Factors during pregnancy influencing development of complex traits in adult life should be expanded to include epigenetic factors, e.g. DNA methylation, which occurs in utero. Epigenetic factors regulate gene expression and thereby cell differentiation and organogenesis. The process of epigenotype establishment is sensitive to environmental conditions, with nutrition being one of the most important factors. For example, DNA methylation depends on availability of several nutrients, including methionine and vitamins B6, B12, and folate.

Conclusion

In summary, poor fetal growth can result from a myriad of fetal, placental, and maternal conditions. Based on numerous studies in various populations, there is a clear association between intrauterine growth restriction and the occurrence of obesity, type 2 diabetes mellitus, cardiovascular, neurological disorders, and reproductive disorders later in life. Animal studies have confirmed that these associations are independent from postnatal influences. However, mechanisms of intrauterine programming and the critical time(s) during pregnancy when an insult causes fetal problems have not been completely elucidated. Although there are treatment modalities available, large-scale prospective studies are needed before therapeutic interventions, given either during pregnancy or after birth, to prevent adverse effects of fetal programming, are scientifically justified. Animal models such as large offspring syndrome, fetal adaptation in mares, placental hypoxic condition in sheep, and nutritional management of IUGR in sheep and pigs, can be used to understand schemes involved in mechanisms and timing of fetal programming, thereby providing new opportunities to reduce or eliminate gestational events which result in fetal, neonatal and adult health shortcomings.

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