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Animal models of fetal growth restriction: Considerations for translational medicine

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ABSTRACT

Fetal growth restriction (FGR) is the failure of a fetus to reach its full genetic growth potential. It occurs in up to 8% of pregnancies, and after premature birth is the second leading cause of infant mortality and morbidity. There is no treatment currently available for FGR. Its primary cause, when not attributable to structural or genetic defects of the fetus, is ‘placental insufficiency’. This broad definition covers the inability of the fetus to acquire sufficient nutrients and oxygen, and is influenced by a number of factors including altered maternal or fetal blood flow, reduced nutrient transport or changes in the placenta such as increased barrier thickness inhibiting nutrient transfer. For those researchers studying FGR and developing new therapies, choosing an animal model is a crucial consideration. It is vital to clearly frame the question being asked, as this will impact the factor influencing fetal nutrient delivery in the model, and will also affect the applicability of the results to the human condition. This review examines the range of in vivo models of FGR available for those engaged in translational research.

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1. Introduction

Fetal growth restriction (FGR) is a failure of a fetus to reach its full genetic growth potential. It occurs in up to 8% of pregnancies, and is the second leading cause of infant mortality and morbidity, following premature birth. Not only are the personal consequences severe, care of these children places a huge financial burden on health care systems, and on social welfare if they survive past infancy. Perhaps more alarmingly, there is no treatment currently available for FGR. When severe and early onset, parents may face the stark choice of delivering a very preterm and possibly non-viable baby, or letting the fetus die in utero. Tables 1–6.

The primary cause of FGR, when not attributable to structural or genetic defects of the fetus, is ‘placental insufficiency’. This is a global term covering the failure of the fetus to acquire nutrients and oxygen adequate for its needs, and is influenced by a number of factors including altered maternal or fetal blood flow, reduced nutrient transport or changes in the placenta such as increased barrier thickness inhibiting nutrient transfer. Asymmetrical FGR can result in severe cases, a compensatory process whereby brain growth is preserved at the expense of other structures such as the liver, abdomen and long bones, a process termed ‘brain sparing’.

When choosing an animal model in which to study FGR and to develop new therapies, it is important to clearly frame the question being asked, as this will have consequences for the factor influencing fetal nutrient delivery in the model, and also for the applicability of the results to the human condition.

2. Why use animal models

We use animal models as the complexity they provide better reproduces the human condition. Some aspects of pregnancy, such as trophoblast development, placentation and placental transport, can be studied in vitro. Human placental villous explants are used to study the materno–fetal interface. The effect of drugs on villous growth and syncytiotrophoblast regeneration can be investigated, or explants from patients with known pathologies can be compared with normal controls for functional studies (reviewed in Ref. [1]). Primary trophoblastic cells isolated from placentas can be cultured short term to study cell function and extrapolate placental remodelling [2,3]. Intricate experimental techniques such as the dual perfusion model of the human placenta [4], where a complete, delivered, human placenta is reperfused in vitro, can be used to look at utero-placental
Table 1
Mouse
Advantages | Disadvantages
---|---
• Small size and social nature thus easy to maintain and relatively inexpensive to house | • Small size means may be problematic to manipulate surgically
• Short gestation reduces the time and expense especially to second and third generation studies | • Imaging of the fetus or placenta can be technically challenging

Table 2
Rat
Advantages | Disadvantages
---|---
• Short gestation, large litters | • More expensive due to size increase over mice
• Large enough for complex surgical intervention | • Altricial young
• Useful for intergeneration studies especially cognitive | |

Table 3
Guinea pig
Advantages | Disadvantages
---|---
• Haemomonochorial placenta | • Longer gestation, larger animal, smaller litters thus more expensive
• Extensive trophoblast invasion | • Less common laboratory animal so specific reagents/equipment more expensive
• Longer gestation, better for therapeutic evaluation | |

Table 4
Other small mammals
Rabbit advantages | Dog advantages
---|---
• Predictable reproductive cycle | • More invasive trophoblast than other domestic animals
• Familiar to regulators for reproductive toxicology | |
• Fetal growth chronologically comparable to human | |

Techniques

haemodynamics and drug transfer but only short term (up to 9 h) before the integrity of the placental barrier is compromised. Currently, this ex vivo technique is the only model available to study organised human placental tissue. Setting up the perfusion experiments is complicated by a high rate of failures due to tissue damage compromising the integrity of the placental barrier. The technique has been used to study placental toxicology and the transfer of drugs and endogenous (eg amino acids and hormones) and exogenous (eg viruses and therapeutics) substrates [5,6]. Dual placental perfusion has also been used in pre-eclampsia to examine the vasodilatory effects of VEGF [7], a
potent angiogenic factor which has also been implicated as playing a role in altered placental angiogenesis in FGR [8,9]. Nevertheless, despite the advances made using in vitro models of some aspects of pregnancy, the condition of FGR as a whole is more accurately represented in vivo. Still other features of pregnancy, such as the development of the uteroplacental circulation, fetal growth velocity and fetal development have no in vitro counterpart. When new therapies become available, although they are not yet tested extensively in vitro, they must present a clean reproductive toxicology panel in vivo before they are deemed fit for use in humans, hence animal experiments are necessary.

### Table 5
Other large mammals.

| Sheep advantages | Sheep disadvantages |
|------------------|---------------------|
| • Serial sampling from both sides of the placental barrier in unaanesthetised and unstressed animal possible | • Placentation is not closely similar to human |
| • Sheep conceptus relevant to human fetal physiology | • Large animal facility needed |
| • Consistent gestation with predominantly singleton pregnancies | • Good tolerance for in utero manipulation |

### Table 6
Non-human primates.

| Intervention | Characteristics | Reference |
|--------------|-----------------|-----------|
| Pig natural runt | Asymmetrical fetal growth restriction, improved cerebral oxygen utilisation | [15,91] |
| Sheep carunculectomy | 30% fetal growth restriction reduction in glucose consumption by placenta and oxygen supply to the fetus, increase in placent al efficiency | [48,92] |
| Sheep nutrient restricted adult | Mild FGR, 17% reduced uterine blood flow, reduced placent al capillary density | [50] |
| Sheep overfed adolescent | FGR, brain sparing, reduced uterine blood flow (36%) and umbilical artery blood flow | [56] |
| Sheep hyperthermia | 46–74% fetal growth restriction with brain sparing reduced uterine and umbilical artery blood flow | [60] |
| Sheep maternal hypoxia | 25% fetal growth restriction with systolic and diastolic fetal cardiac dysfunction | [93] |
| Goat nutrient or protein restriction | 5–10% fetal growth restriction altered fetal thymus, small amount of liver and kidney size relative to body weight | [94] |
| Horse crossbreed | IVF experiments in Shetland ponies and thoroughbred racehorses, fetus is constrained by the size of the surrogate mother | [95] |

3. Considerations when studying animal pregnancies

There are a number of species-specific factors to be considered when using animals to model human pregnancy. The number of offspring per pregnancy, placental growth, gestation length, parturition and fetal versus neonatal development will all affect the choice of model.

#### 3.1. Fetal number

A large litter size has the advantage of acquiring good quality data from a minimal number of pregnancies, reducing the overall number of animals required. Differences between the sexes can also be studied more easily. Additionally, animals with large litters such as rabbits and pigs have a ‘natural’ model for FGR in the runt of the litter. These animals can also be ‘forced’ into creating runts where a normal size litter is carried in a single uterine horn following a unilateral ovariectomy [11,12]. For rabbits, pups in the middle of the uterine horn are consistently smaller than their littermates, as they are further away from the blood supply arriving via ovarian or cervical ends of the uterine artery. In a natural rabbit model of FGR, when compared to kits in the ‘favour ed’ positions nearer the arterial source, runts are consistently smaller. At term, the weight ratio of favoured to FGR fetus is 0.85 [13,14]. They also have depressed liver, kidney, and intestinal expression of insulin growth factor 1 (IGF-1) mRNA as well as lower serum and amniotic fluid levels of IGF-1 protein, a key component in modulating fetal growth that is reduced in the cord blood at term of human babies with FGR [14]. Piglet runts spontaneously display asymmetric growth restriction [15]. For direct fetal treatment however, a large number of fetuses can make intervention technically difficult. Fetal measurements can also be challenging and time-consuming, and interventions may have a prolonged anaestheti c effect. There are also statistical considerations to be taken into account with litters. Pup birth weight varies with position, watershed area, sex, number, gestational age at delivery and intervention. Importantly, when considering interventions given to mothers, the mother is the unit of measurement and pups are nested within mothers. Sample size calculations and analysis of the primary and secondary outcomes in a study must account for all these factors to be able to see the true effect of an intervention.

#### 3.2. Length of gestation

Small animals, such as rodents and rabbits, tend to have short gestation lengths. Much of the development that would take place during fetal life in the human occurs in the neonatal period in these animals. This makes them less relevant as models for the consequences of FGR in the neonate, especially with regard to neuro-developmental impact. However, their shorter lifespan is an advantage for the study of trans-generational effects, significantly reducing the time needed to gather data. Among rodents the guinea pig is an exception, having a comparatively long gestation, but there is a lack of genetic models of disease in this species. A long gestation length can also confer considerable advantages. There is an increased time in which to evaluate the effect of a therapy or intervention on multiple parameters such as fetal development, fetal growth, and miscarriage rates. These advantages must be weighed against the cost of maintaining animals over the longer period, finally coming down to a justification of the cost of the model versus the quality and type of data that it will provide.

#### 3.3. Placental shape

Placenta is a complex process, and as the majority of FGR arises from placental insufficiency there are several aspects of
placenta, so the mouse would not be a suitable model for investigation into the causes of inadequate spiral artery remodelling, nor any interventions aimed at promoting trophoblast invasion. In contrast, the guinea pig has extensive trophoblast invasion, which spreads deep into the walls of the uterine arteries [32,33]. The sheep, as with other ruminants, has no trophoblast invasion, and while the maternal vessels in the rabbit are lined with multinucleated cells it has not been established that these are trophoblastic [30]. The canine placenta is more invasive than other domestic animal models, however it more closely resembles a human pre eclamptic transformation than a normal pregnancy [34]. As the nearest genetic relative to the human, it is unsurprising that in non human primates the remodelling of the spiral arteries following intervention in these animal models therefore needs to consider this aspect when trying to translate into the clinical situation.

3.7. Trophoblast invasion

The extent to which fetal trophoblast invade the maternal tissue and remodel the spiral arteries is also an important consideration. No other organism has such extensive invasion as humans, and both the extent and depth of trophoblast invasion is suboptimal in FGR [29]. Trophoblast invasion is shallow in most rodents, with rats having more extensive invasion than mice, where uterine artery transformation is more dependent on maternal factors such as natural killer cells, than on trophoblast invasion [30,31]. In humans, trophoblast invasion is crucial to adequate supply of blood to the placenta, so the mouse would not be a suitable model for investigation into the causes of inadequate spiral artery remodelling, nor any interventions aimed at promoting trophoblast invasion. In contrast, the guinea pig has extensive trophoblast invasion, which spreads deep into the walls of the uterine arteries [32,33]. The sheep, as with other ruminants, has no trophoblast invasion, and while the maternal vessels in the rabbit are lined with multinucleated cells it has not been established that these are trophoblastic [30]. The canine placenta is more invasive than other domestic animal models, however it more closely resembles a human pre eclamptic transformation than a normal pregnancy [34]. As the nearest genetic relative to the human, it is unsurprising that in non human primates the remodelling of the spiral arteries following trophoblast invasion most closely resembles the human condition [35,36]. Even so, there remain some differences between the species, such as a relative lack of interstitial trophoblast cells in non human primates.

4. Creating animal models of fetal growth restriction

Animal models of FGR fall into three broad categories when divided by method of intervention creating the model: fetal intervention, maternal intervention, and genetic models. For each commonly used species we list a selection of FGR models in Tables 1 – 6. The primary model using fetal intervention is the hypoxic chick [37–39]. The principal advantage of this model is the ability to investigate the effects of hypoxia in the fetus in isolation, without affecting the mother. In many growth restricted human pregnancies caused by placental insufficiency, there is no alteration
in the health status of the mother. Alternative fetal interventions include infection with certain viruses and dosing with radioactive iodine, however these are not useful for translational medicine.

There are a range of maternal interventions capable of creating FGR in an animal model. The oldest interventions directly alter the uterine circulation, reducing maternal nutrient and oxygen transport to the placenta. Uterine artery ligation has been shown to cause FGR in rats, guinea pigs, and sheep [40–44]. Similarly, radial artery diathermy in guinea pigs and uterine artery embolization in sheep also result in FGR [45–47]. Although the result is FGR, the lack of an intact utero-placental circulation in these models renders them less useful for testing maternal therapies that target uterine blood flow or the placental barrier directly. A related intervention is sheep carunclectomy, in which the maternal portion of the placentomes — the multiple contact points between maternal and fetal blood circulations in the placenta — are surgically removed from the uterus prior to pregnancy. This creates FGR in about half of pregnancies [48], illustrating the relative redundancy of the sheep placenta.

Global interventions in the mother are also able to cause FGR in pregnancy. In humans, a major reduction in calorie intake is needed to influence fetal weight, such as occurred in the World War II Dutch famine, and the effect is dependent on the trimester of gestation in which it occurs [49]. As is to be expected, maternal nutrient restriction results in smaller fetuses with asymmetrical growth in several models, including rat, guinea pig, rabbit, and sheep [50–55]. Interestingly, overfeeding in an adolescent pregnant ewe also causes FGR, as growth of the mother is maintained at the expense of fetal development [56]. Alternatively, restriction of specific nutrients such as in low protein or low sodium diets, rather than overall calorie reduction, can also impact the growth of the fetus in several models [57–59]. Environmental factors are also capable of influencing pregnancy outcome. Following observations of low birthweight lambs from sheep raised in hot conditions, a heat stress model of FGR was developed [60]. Fetuses showed evidence of brain sparing, and umbilical and uterine blood flow was reduced [60,61]. The hyperthermic conditions were able to be imposed at various time points, and highlighted that the timing of intervention used to create the FGR model can have a large impact on the resulting disease phenotype. Finally, pregnancy at high altitude or a period spent in a hypoxic chamber, limiting the oxygen supply to the mother and thereby to the placenta and fetus, can cause FGR, though this varies by species and again is dependent on the gestational timing of the insult. Using animals that are native or naïve to high altitude mimics the effects seen in human pregnancy, in which compensatory mechanisms such as altered enzymatic antioxidant activity may contribute to native protection from some of the consequences of a reduced oxygen tension [62].

Genetic models of FGR have generally been created in the mouse (recently reviewed in Ref. [63]), aided by the wealth of molecular information available in that species and ready access to embryonic stem cells. Early knockout models proved to be overly severe, and resulted in embryonic lethal phenotypes. Global disruption of Tissue Factor, also known as platelet tissue factor, factor III, thrombokinase, or CD142, resulted in fatal wasting of mouse offspring after embryonic day 9.5 [64]. Unconditional knockout of vascular endothelial growth factor (VEGF), a signal protein which is essential for angiogenesis during development, also produces an embryonic lethal phenotype [65]. More refined models are now available, with conditional or tissue-restricted knockout of specific genes. In the placental specific insulin-like growth factor 2 (IGF2) knockout mouse model, a transcript of the gene which is expressed only in the placental labyrinthine trophoblast cells is deleted [66]. This results in impaired placental growth from embryonic day 12, and growth restriction in 96% of fetuses by embryonic day 16. Birth weight is approximately 69% of wild type, although the pups did exhibit postnatal catch up growth. Impaired placental growth is seen earlier in gestation than reduced fetal growth, possibly as a result of increased placental System A activity that may contribute to maintaining fetal growth. Closer to term, the knockout placentas remain smaller, the System A activity is nearer to normal and there is decreased passive permeability as well, all of which likely contribute to the FGR phenotype [66]. A global knockout of the endothelial nitric oxide synthase (eNOS) gene, an enzyme which converts arginine to nitric oxide (NO) inducing vasodilation, results in impaired uterine artery function and diminished placental System A amino acid transporter activity [67]. This model shows asymmetric growth and a possible reduction in extraction of oxygen by the fetus [67]. In humans, the level of system A activity is correlated with severity of FGR [24].

For all animal models, using a standardized method to describe and express fetal growth is important. An examples of such a method includes constructing fetal growth curves [68], which allows comparison of animal data with human FGR data, and to quantify how much of an improvement in fetal growth an intervention might achieve in the clinic.

5. Translational medicine considerations

The practicalities of testing out drugs and therapies in animal models of FGR present numerous challenges, both applied and regulatory [69]. Since many models require an initial surgical intervention, a further surgical intervention may be deemed too stressful for the animal and may not be allowable under animal experiment regulations governing specific countries, or may render insufficient numbers of pups for evaluation. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines govern reproductive toxicity studies in women and men [10], specifying that any programme should allow exposure of the novel chemical to all stages of development throughout one complete life cycle: for example, from conception in one generation through to conception in the following generation. Where more than one investigation is used there must be an overlap between studies so that no gaps are left between key stages. This is especially relevant if a drug affects fertility, when there may not be adequate numbers of pregnant animals or fetuses to properly assess developmental toxicity in utero. In practice, a number of overlapping studies are conducted to cover fertility and early embryonic development, pre- and postnatal development including lactation and weaning, and embryo–fetal development [70]. All studies need to be conducted under Good Laboratory Practice (GLP) conditions, which are extremely costly. Fertility and pre- and postnatal development studies need only be conducted in one mammalian species, which is commonly the rat. For embryofetal development two mammalian species should usually be tested, one should be a rodent, often the rat for pragmatic reasons, and the other a non–rodent, usually the rabbit where there is a large body of historical data for comparison. Alternative species may be considered if there are good reasons such as specifics relating to drug metabolism, although for some species, there may not be much historical data, making interpretation of results challenging (J Baldwin, personal communication).

A translational medicine study is in essence a clinical trial but performed in animals with parallel considerations. Generating the best control group may require collection of local contemporaneous data in the same species without the intervention that created FGR, since relying on historical data may introduce bias. The type of control group is important, whether they be untreated, have a sham intervention or use a control treatment administration. The
advantage of an animal study is that controls can be selected for their appropriateness rather than what is permissible in a patient. Clinical trials are run according to set principles (eg UK Clinical Trials Regulations 2004, EU Clinical Trials Directive 2001), and animal studies should follow similar principles to provide the most robust data. Interventions should be adequately described, study groups should be randomly allocated and blinded where possible, sufficiently powered, with pre-specified clinically important endpoints and results reported together with important adverse events. One example, the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [71], were developed as part of an initiative by the UK National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs). The guidelines are intended to improve the design, analysis and reporting of research using animals by maximising the information that is published and minimising unnecessary studies, and have been adopted by many scientific journals.

Additionally relevant to FGR, consideration needs to be given to methods of monitoring fetal loss and growth. Serial measurement in one animal may yield more information than multiple post mortem sampling in a number of different animals. In particular measurement of uterine blood flow is feasible in large animals using ultrasound or implanted flow probes and may yield valuable longitudinal data after intervention [72–74]. Ultrasound allows non-invasive longitudinal monitoring, although it does require skilled technicians, who should be blinded to the intervention where possible [75]. Care should be taken when assessing whether pups are resorbed or not, especially with regard to whether this is due to the intervention/therapy or the creation of FGR.

If animals are going to deliver their offspring there are further considerations. FGR animals often have a higher rate of pregnancy complications, leading to higher fetal and perinatal loss rate. The offspring in some models are very fragile at birth and may be preferentially cannibalised by rodents, or need sustained intervention in order to thrive, some even occasioning an animal "neonatal intensive care unit". Measuring interventions of this nature accurately is challenging. There may be an effect of the creation of FGR on the ability of mother to feed offspring. Among other examples, some FGR ewes have very poor lactation initially [14]."

**Conflict of interest**

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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