Placenta 36 (2015) 623–630

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Current opinion

Animal models of fetal growth restriction: Considerations for translational medicine

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ARTICLE INFO

Article history:
Accepted 4 March 2015

Keywords:
Fetal growth restriction
Animal models
Translational medicine

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.

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

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http://dx.doi.org/10.1016/j.placenta.2015.03.003

Table 1
Mouse.

Advantages	Disadvantages
• Small size and social nature thus easy to maintain and relatively inexpensive to house • Short gestation reduces the time and expense especially to second and third generation studies	• Small size means may be problematic to manipulate surgically • Imaging of the fetus or placenta can be technically challenging • Can be difficult to follow serially postnatally, due to cannibalization and the challenge of marking newborn mice • Differences between human and mouse physiology, though generally well characterized and understood • Altricial young

Intervention	Characteristics	Reference
Erk3$^{-/-}$	Fetal growth restriction with 25–40% reduction of visceral organ growth 40% die at birth from acute respiratory failure, similar to respiratory distress syndrome in humans	[77]
VEGF knockout	Homozygous is embryonic lethal heterozygous results in fetal growth restriction	[65]
eNOS$^{-/-}$	Fetal growth restriction with brain sparing, hypoxia and reduced placental system A transport	[67]
Placental specific IGF2$^{-/-}$ (P0)	30% fetal growth restriction with brain sparing	[66]
Protein restriction	Fetal growth restriction with adiposity	[58]
'Crowded uterine horn'	Unilateral ovariectomy pre-pregnancy producing a normal size litter in single horn Differential blood flow results in fetal growth restriction in the middle fetuses	[11]

Table 2
Rat.

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

Intervention	Characteristics	Reference
Uterine artery ligation	40% fetal growth restriction with brain sparing high level fetal loss and resorption	[44]
Uterine artery occlusion (60 min)	Fetal growth restriction with brain sparing fetal mortality 14%	[78]
Dexamethasone administration	15% fetal growth restriction chronic hypertension in adult offspring	[79]
L-NAME administration	Fetal growth restriction up to 20%, increased stillbirth dependent on dose regimen	[80,81]
Hypoxia	Both chronic and intermittent hypoxia in second half of pregnancy effective 4–37% fetal growth restriction, varied level of exposure	[82–84]
Nutrient restriction	Fetal growth restriction up to 35% both acute fasting and chronic restriction effective significant changes to the IGF axis in offspring	[51,55,85]
Protein restriction	15% fetal growth restriction	[59]

Table 3
Guinea pig.

Advantages	Disadvantages
• Haemomonochorial placenta • Extensive trophoblast invasion • Longer gestation, better for therapeutic evaluation • Precocial young thus brain development more like human than other rodents	• Longer gestation, larger animal, smaller litters thus more expensive • Less common laboratory animal so specific reagents/equipment more expensive

Intervention	Characteristics	Reference
Uterine artery ligation	40–60% fetal growth restriction in a proportion of fetuses High rate of fetal death Reduced oxygen and nutrient delivery to fetus	[42,86,87]
Radial artery diathermy	30% fetal growth restriction with brain sparing reduction in brain growth, both volume and neuronal number	[46]
Maternal nutrient restriction	Both acute fasting and chronic restriction effective 10–39% fetal growth restriction with brain sparing altered trophoblast density, placental barrier thickness	[52,54]

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

Table 4
Other small mammals.

Rabbit advantages	Dog advantages
• Predictable reproductive cycle • Familiar to regulators for reproductive toxicology • Fetal growth chronologically comparable to human	• More invasive trophoblast than other domestic animals

Intervention	Characteristics	Reference
Rabbit natural model	Based on fetal position in the uterine horn 15% fetal growth restriction Depressed expression of IGF-1 mRNA and lower serum and amniotic fluid levels of IGF-1 protein	[14]
Rabbit thermal placental injury	30% fetal growth restriction with brain sparing	[88]
Rabbit high cholesterol diet	15% fetal growth restriction	[89]
Rabbit selective uterine artery ligature	28% fetal growth restriction increased fetal mortality compared to undernutrition Doppler parameters more closely reproduce human FGR	[50]
Canine acute nutrient restriction	10% fetal growth restriction alterations in fasting glucose and fat metabolism	[90]

Table 5
Other large mammals.

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

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 placental efficiency	[48,92]
Sheep nutrient restricted adult	Mild FGR, 17% reduced uterine blood flow, reduced placental 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 systolic and diastolic fetal cardiac dysfunction	[93]
Goat nutrient or protein restriction	5–10% fetal growth restriction altered fetal thymus, small intestine, kidney and liver weights 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]

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 first tested extensively *in vitro*, they must present a clean reproductive toxicology panel *in vivo* [10] before they are deemed fit for use in humans, hence animal experiments are necessary.

Table 6
Non-human primates.

Advantages	Disadvantages
• Genetically, closest model to human • Pregnancy characterised by trophoblast invasion of the spiral arteries	• High cost and dedicated facility needed • Ethical considerations • Study numbers generally small • Interventions cause high rates of fetal loss

Intervention	Characteristics	Reference
Baboon nutrient restriction	10% fetal growth restriction, changes to fetal brain, liver, kidney, placenta	[96]
Rhesus macaque ligation of placental bridging vessels	6–14% fetal growth restriction with asymmetrical growth, dependent on time of insult, 40% reduction in functional placenta	[97,98]

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, placentation, 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 'favoured' 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 anaesthetic time. 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 neurological 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

Placentation is a complex process, and as the majority of FGR arises from placental insufficiency there are several aspects of

placentation which need to be considered. The human placenta is discoidal in shape, as it is in higher primates and rodents, with a single disc-like zone of close maternofetal contact. This provides the highest concentration of maternofetal interdigitation, an intricate system of folds which increases the area of contact between mother and fetus. Lower primates and pigs have a diffuse placenta, where the interdigitation is distributed over the entire maternofetal exchange area. Ruminants, including sheep, have a cotyledonary placenta, in which many spot-like regions of intense maternofetal interdigitations exist.

3.4. Interdigitation

Interdigitation can be further sub-divided into five types [16]. Folded interdigitations are the most simple, with ridge-like folds of the chorion that fit into corresponding grooves of the uterine mucosa, and are found only in animals which have a diffuse placenta, such as pigs. Lamellar interdigitation is more complex, with ridges branching into parallel chorionic lamellae interspersed with branched endometrial folds, and is seen in some types of carnivore. The trabecular type has interdigitations from which leaf-like and finger-like villi branch, and has been described in some monkeys. Sheep, humans and other higher primates have villous interdigitation, where the chorion has a tree-like branching pattern, and villi either fit into endometrial crypts or are directly bathed in maternal blood. The final and most common type is labyrinthine, found in rodents, where a trophoblastic mass is permeated by a network of channels filled with maternal blood or fetal capillaries.

3.5. Interface

The type of maternal–fetal interface [16] present is important for passive transfer of oxygen across the placenta. Across species there appears to be a relationship between the number of cell layers in the placental exchange barrier and permeability [17]. In the synepitheliochorial placenta for example, such as found in ruminants, there are six layers of tissue between maternal and fetal blood [18]. This compares to one syncytiotrophoblast layer in the late gestation haemomonochorial human placenta. Perfusion experiments and calculations suggest that the permeability of the sheep placenta is at least one order of magnitude less than that of the human [19]. In an endotheliochorial placenta, seen primarily in carnivores, invading trophoblasts face the maternal endothelium and only five tissue layers separate the maternal and fetal circulation. In a haemochorial placenta, the trophoblasts also erode the maternal vessels and so maternal blood bathes the syncytiotrophoblast and there are just three tissue layers between maternal and fetal blood. Depending on the number of trophoblastic epithelial layers a more detailed subdivision has been proposed, which in humans depends on the gestational age: haemotrichorial (rat and mouse), haemodichorial (rabbit and human in the first trimester), and haemomonochorial (great apes, guinea pig and human at term). This has implications for drug testing in animals. For example, if placental transfer of a drug intended for early pregnancy were being investigated, a rabbit model may be preferred. If the drug was to be administered only in late pregnancy, a guinea pig model may be more appropriate.

3.6. Placental transport

A maternal–fetal counter current arrangement is deemed the most efficient anatomical arrangement of blood vessels, yet placentas with counter current flows, such as the guinea pig [20], rat and rabbit are not more efficient than other types, such as crosscurrent in the sheep, where fetus produced per gram placenta (a measure of placental efficiency) is equivalent [21]. Facilitated and active transports are important to overall placental efficiency, as is the total surface are available for diffusion. Nutrients such as glucose and lactate are transferred across the placenta by facilitated transport via transporter proteins. There are several isoforms of the glucose transporter, which are differentially distributed in the human placenta, and vary from distribution in other animals (reviewed in Ref. [22]). Active transport of essential amino acids and ions such as sodium and potassium is mediated by a range of specific transporters, and the activity of these transporters may also differ between species [23]. Alterations in the activity of certain nutrient transporters have been seen in human FGR [24,25] and some animal models of FGR [23,25–27]. Transport of proteins across the placenta is largely restricted to proteins of specific use to the fetus, such as immunoglobins, although this is confined to hemochorial placentas. Active transport of immunoglobulins occurs via the placenta in humans, and via the yolk sac in rodents and rabbits [28]. In contrast to the human placenta, epithelio- and endotheliochorial placentas are impermeable to proteins, and immunoglobins are transferred in the colostrum after birth [16]. Examination of the maternal and/or fetal immune response to fetal interventions 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 uteroplacental 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 pregnancy 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 and neonatal lambs may need supplemental colostrum [76]. When complications occur, they should be fully investigated, with a detailed post-mortem examination, blood analysis, histology and microbiological analysis where feasible. Evaluation of important long term outcomes such as cardiovascular disease, hypertension, insulin sensitivity and even the F1 generation will need sufficient initial animal numbers to account for these losses. Finally, and by no means to be considered last, are the statistical analysis to evaluate the results which must include consideration of confounding variables (mother weight, litter number, pup sex, pup position etc).

6. Conclusion

Developing new therapies for fetal growth restriction ultimately requires the use of animal models in which to test efficacy and safety. The choice of which animal to use will need to take into consideration the characteristics of pregnancy in the particular animal, and whether to use naturally occurring FGR or FGR created by maternal or fetal intervention, or genetic manipulation. 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. Use of guidelines, such as ARRIVE, will improve the quality of the study and minimise unnecessary follow-up studies.

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.

Acknowledgements

ALD is funded by a HEFCE/Department of Health Clinical Senior Lectureship. AMS is funded by Action Medical Research (GN1738) and the Rosetrees Trust (SP4409). This work was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health NIHR Biomedical Research Centre's funding scheme.

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