Placenta directed gene therapy for fetal growth restriction (A)

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Abstract (B)

Fetal growth restriction (FGR) is a serious pregnancy complication affecting approximately 8% of all pregnancies. There is no treatment to increase fetal growth in the uterus. Gene therapy presents a promising treatment strategy for FGR, with the use of adenoviral vectors encoding for proteins such as Vascular Endothelial Growth Factor (VEGF) and Insulin-Like Growth Factor (IGF) demonstrating improvements in fetal growth, placental function and neonatal outcome in preclinical studies. Safety assessments suggest no adverse risk to the mother or fetus for VEGF maternal gene therapy; a clinical trial is in development. This review assesses research into placenta directed gene therapy for FGR, investigating the use of transgenes and vectors, their route of administration in obstetrics, and the steps that will be needed to take this treatment modality into the clinic.

Key Words (B)

Fetal growth restriction (FGR), Vascular endothelial growth factor (VEGF). Insulin-Like Growth Factor (IGF), Gene therapy, Transduction, Vector, Adenovirus, Placenta, Uterine artery

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INTRODUCTION (B)

Gene therapy allows for the transfer of genetic material into a target cell with the aim of achieving therapeutic benefit. Since the first gene therapy trials in the 1990s, there has been hope that gene therapy could improve the management and outcomes of genetic diseases, particularly single-gene disorders. There are currently over 1800 completed or on-going gene therapy clinical trials, of which over two-thirds are on cancer. Gene therapies are now becoming licenced for use in the clinic, the first being Gendicine™ in 2003 in China, for the treatment of head and neck squamous cell carcinoma, closely followed by Oncorine™, a p53 gene therapy for late-stage nasopharyngeal cancer in 2005 (1). In 2012 Glybera™, a treatment for familial lipoprotein lipase deficiency became the first gene therapy product to be approved for licensing in Europe (2).

Pre-clinical studies show that adeno-associated viral vector gene therapy to the fetus can cure single-gene disorders such as haemophilia. Concerns about germline gene transfer and off-target effects in the fetus however, are holding back translation into the clinic of fetal-directed gene therapy(3). Serious maternal obstetric diseases such as pre-eclampsia and fetal growth restriction (FGR) also affect the fetus, and neonate long term. Understanding the molecular basis of these untreatable obstetric diseases has lead to an understanding of the potential role that placental directed gene therapy could play (4). This review will consider the application of gene therapy to the placenta to treat fetal growth restriction. Results of pre-clinical studies are compelling and clinical trials are being planned. The limitations and risks of gene therapy in the maternal setting will be evaluated, and the current ethical and regulatory issues will be presented.

FETAL GROWTH RESTRICTION (B)

Optimal fetal growth depends on functioning maternal, placental and fetal factors, the external environmental, in conjunction with a genetically pre-determined growth potential. Fetal growth restriction (FGR) can occur due to a malfunction of a single or number of these factors. It is potentially life threatening and affects 8% of all pregnancies, contributing to 50% of stillbirths (5). Of those diagnosed with FGR, approximately 1 in 500 cases are classified as both severe and early onset, occurring before 28 weeks of gestation. Some severe FGR is caused by structural abnormalities of the fetus, maternal medical disorders and congenital infections. Most commonly, impaired uteroplacental function restricts delivery of nutrients to the fetus, resulting in slowing or even cessation of fetal growth, termed placental insufficiency.

In normal pregnancies, effective first-trimester infiltration of the trophoblast in the maternal spiral arteries leads to the creation of a high flow, low resistance maternal circulation. Angiogenesis and vasodilation in the placenta are enhanced by the production of factors such as placental growth factor (PlGF), vascular endothelial growth factor (VEGF) and insulin-growth factor (IGF) (6, 7), which facilitates a reduction in placental resistance. The obstetric syndromes of pre-eclampsia and FGR appear to be interrelated through VEGF biology. An increase in soluble fms-like tyrosine kinase 1 (sFlt1), which acts as a soluble receptor for VEGF in the maternal circulation, is observed in both conditions (8, 9). Treatments based on the manipulation of VEGF and related angiogenic factors are therefore likely to be effective for FGR and pre-eclampsia.

When FGR is severe and early in onset, management involves prompt delivery of the fetus before death or irreversible organ damage occurs, particularly to the brain. However, delivering the fetus in severe early onset FGR adds additional risks to the baby from extreme prematurity (10). In this situation the question of viability also arises, and decision making with parents is challenging (3). Substantial improvements in morbidity and mortality can be seen if delivery of such pregnancies can be delayed by even one week (e.g. from 26 to 27 weeks) and if there are modest increases in birth weight (eg 100g) (11). It is in these severe early-onset cases of FGR that maternal gene therapy is initially being considered, where the benefit of gaining gestation or improved fetal weight might

outweigh the potential risks of a novel therapy. If it is found to be safe and efficacious there is potential to use maternal gene therapy in more moderate FGR, which affects a larger number of pregnancies.

WHAT IS GENE THERAPY? (B)

Gene therapy is the transfer of genetic material to targeted cells into order to modify or treat a disease. To produce a therapeutic result, genetic information, otherwise known as a transgene, is introduced using a vector into the target cells. The transgenic protein that is produced by transcription of the transgene generates a therapeutic effect. More recently gene editing has emerged as a potentially more targeted form of gene therapy whereby a nuclease cuts the DNA helix to create a specific double-stranded break, which is then repaired using template DNA to enable the production of a therapeutic protein(12).

Somatic or stem cell gene therapy is applied directly to target organs or cells, but does not cause multi-generational effects. Germ line therapy however, will be passed onto future generations which current legislation precludes. Transgenes consist of several elements; a promoter, which regulates when and how the transgene is activated; an exon, the part of the gene that encodes mature RNA to produce a coding sequence; and finally a intron, which acts as a transcriptional stop sequence. Gene therapy has proved to be successful in single gene disorders, whereby a missing or defective gene can be replaced, as in the case of haemophilia, ß-thalassemia and X-linked severe combined immunodeficiency. Achieving safe, long-term expression continues to prove a challenge in monogenic disorders (2). Within cardiovascular disease, VEGF gene therapy is being applied to coronary artery disease(13).

Types of vector (C)

The choice of vector is critical in gene therapy. Manufacture of the vector would ideally be simple and cost-effective, it should be capable of being targeted to the specific tissue or organ and generate a transgenic protein for the required length of time to have a therapeutic effect without causing side effects (14). For obstetric conditions such as FGR and placental insufficiency, the

therapeutic time frame would be short, limited by the length of gestation. When targeting specific organs such as the placenta, the method of delivery can have considerable impact on the level and site of genetic expression.

The most commonly tested vectors in fetal gene therapy preclinical studies have been adenovirus and adeno-associated virus, lentivirus and retrovirus vectors. The characteristics of these and other less commonly used vector systems are described in Table 1. Manipulating the vector structure and the transgene can alter vector properties. Pseudotyping for example, involves changing the virus capsid (outer covering) for one of a different serotype or of a completely different virus thus altering its ability to infect particular cell types or organs(15). Using alternative enhancer-promoters can improve gene transfer to specific organs or tissues, and can even be manipulated to allow regulatable gene expression if required(16). Many replication-deficient lentiviruses are based on the immunodeficiency virus, with a theoretical possibility of reversion to the wild-type. In 3rd and 4th generation lentivirus vectors however, the risk of *in vivo* generation of replication competent viruses is reduced by removal of the *tat* gene. Modification of virus elements, such as mutating the integrase in lentiviral vectors, renders it incapable of integrating and greatly reduces the risk of insertional mutagenesis (17). Clinical grade production of vectors are tested rigorously for replication competent viruses.

Gene editing is an attractive alternative approach to correcting gene defects, which avoids the use of vectors to introduce therapeutic transgenes(12). Reports of successful applications to genomic targets are appearing at an accelerating rate. RNA-guided engineered nucleases (RGENs) derived from the bacterial clustered regularly interspaced short palindromic repeat (CRISPR)-Cas (CRISPR-associated) system are now available that give high-precision genome editing. This would avoid the introduction into the cell or genome of the extra DNA or RNA components that viral or non-viral vector approaches require. Stem cell gene editing is already being applied in the clinic, but somatic gene editing, as would be needed for placenta targeting, is likely to take longer to reach the clinic however, as more long-term safety assessments will be needed.

MATERNAL GENE THERAPY (B)

Maternal gene therapy aims to facilitate expression of proteins in the mother that have translational benefits to the fetus, given the antenatal interdependence between a mother and fetus. Only short-term gene expression would be required to manage obstetric disease, and the concerns surrounding monogenetic and oncologic conditions requiring long-term gene therapies would not apply.

Vascular Endothelial Growth Factor (C)

VEGF stimulates angiogenesis and vasculogenesis, and plays an important role in hypoxic conditions where circulating blood flow is insufficient. Overexpression of VEGF can also cause diseases such as cancers, where improvements in blood circulate promote tumour growth. In mammals, five of sevens known proteins within the VEGF family occur naturally, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). Exon splicing of the gene that encodes VEGF-A creates five molecular variants, differentiated by their amnio acid count, of which the most predominant form is VEGF-$_{165}$. Angiogenic effects have been observed in VEGF-A and VEGF-D; VEGF-D can be pre-processed to a shorter more highly active VEGF-D$^{\Delta N \Delta C}$ isoform(18). Three known VEGF receptors VEGFR-1, VEGFR-2 and VEGFR-3 bind proteins in order to generate angiogenic and lymphangiogenic effects (19). Vasculogenesis is reliant on VEGF-A, with inactivation of even a single allele resulting in dysfunctional vascular development and even embryonic death (20, 21).

Experiments in pregnant sheep and guinea pigs have demonstrated that adenovirus vectors containing VEGF isoforms A_{165} (Ad.VEGF-A_{165}) or pre-processed D isoform (Ad.VEGF-D$^{\Delta N \Delta C}$) increase uterine blood flow and fetal growth in normal and FGR pregnancy. In sheep, mid-gestation injection of the Ad.VEGF-A_{165} vector into the uterine artery via laparotomy was compared with the effect of contralateral uterine artery injection of a control ß-galactosidase (Ad.LacZ) vector containing a non-vasoactive transgene LacZ. Uterine artery blood flow was significantly increased in the uterine arteries treated with Ad.VEGF-A_{165}, both short (4-7 days) and long term (28-30 days) after vector administration, with an associated increase in vasorelaxation and a reduction in vascular contractility short term (22, 23). There was also short term up-regulation of VEGFR-2 and endothelial nitric oxide synthase (eNOS) in Ad.VEGF-A_{165} treated arteries compared with control and non-transduced vessels. Perivascular adventitial endothelial cell proliferation increased in uterine

arteries treated with Ad.VEGF-A_{165} short term when compared with control vessels. Histologically an inflammatory infiltrate was seen in these Ad.VEGF-A_{165} transduced arteries. Human VEGF was undetectable by 7 days after vector injection, suggesting that efficacy of VEGF gene therapy extends beyond transgenic protein expression, a likely secondary result of vascular remodelling (22-24). Results were similar with the Ad.VEGF-$D^{\Delta N \Delta C}$ vector. Importantly there was no evidence in the Ad.VEGF-$D^{\Delta N \Delta C}$ treated vessels of the previously seen inflammatory infiltrate suggesting that this vector has a more favourable immunological profile. when compared to the Ad.VEGF-A_{165} vector (24).

Ad.VEGF-A_{165} treatment was tested in the overnourished sheep model of FGR. Herein adolescent ewes receive a high dietary intake, which causes accelerated maternal tissue growth at the expense of the fetus with an associated mid-gestation decrease in uterine artery blood flow by approximately 42% (25). The model thus replicates the fundamental clinical features of uteroplacental dysfunction: impaired placental vasculogenesis, increased umbilical artery resistance on Doppler ultrasound, fetal brain sparing secondary to asymmetrical growth and reduced placental expression of VEGF (26). Mid-gestation, these overnourished adolescent FGR ewes were randomised to receive either Ad.VEGF-A_{165} or a control treatment (Ad.LacZ or saline) that was directly injected in both uterine arteries. Results are summarised in Figure 1. At 3 and 4 weeks following treatment, serial ultrasound fetal measurements demonstrated an increase in abdominal circumference in Ad.VEGF-A_{165} group when compared with the control (27, 28), with reduced evidence of brain sparing. At term gestation there were significantly fewer severe FGR fetuses in the Ad.VEGF-A_{165} group compared to the two control groups. In a separate experimental cohort, where pregnancies continued until spontaneous delivery occurred, there was ongoing accelerated weight gains observed postnatally without detrimental effects on histology (29, 30).

Whilst the sheep model is useful for obstetric studies given their similarities to human with regards to conception, gestation and fetal physiology, the guinea pig has advantages for FGR studies due to the placentation, trophoblast invasion and cell proliferation to the human placenta (31-33). Periconceptual nutrient restriction in guinea pigs impairs placental growth and function and alter placental exchange with the trophoblast, causing a reduction in fetal weight with brain sparing by

mid-gestation (34, 35). Ad.VEGF-A_{165} treatment of the uterine and radial arteries via laparotomy in mid-gestation guinea pigs at 30-34 days following conception improved fetal growth at term and a lower brain: liver weight ratio, implying that brain sparing had been partly mitigated (36, 37). The improvements in fetal birth weight and uterine artery blood flow in FGR-animal models treated with Ad.VEGF-A_{165} establish VEGF as a transgenic factor with therapeutic benefit in FGR-affected pregnancies, making it a strong candidate for translation into human clinical trials.

Insulin Growth Factor (C)

Targeting placental function is another potentially important therapeutic target in the treatment of FGR. Insulin-like growth factors (IGFs) are important for cell proliferation and apoptotic inhibition. Within the IGF complex are three ligands, four receptors and six IGF binding proteins (38). IGF-I and IGF-II is expressed in human placental villous mesenchymal cells, and throughout gestation in rodents and humans. IGF-I and IGF-II promote placental cell proliferation and survival, and facilitate the placental uptake of glucose and amino acids (39). In contrast, deletion of the IGF-II gene in rodents causes abnormal placental growth and FGR (40). In humans IGF-I deficiency is linked to severe FGR and dysfunctional neurodevelopment (41, 42).

In chronically catheterised FGR sheep, continuous infusion or thrice weekly infusions of IGF-I into the amniotic cavity increase fetal growth rate(43). Long term, IGF levels could be increased through gene therapy. *In vitro* experiments using human placental fibroblasts transduced with an adenoviral vector containing either IGF-I (Ad.IGF-I) or IGF-II (Ad-IGF-II) demonstrated increased mitogenesis when compared with controls (44). Further *in vitro* studies observed increased IGF-I secretion, proliferation, invasion and amino acid uptake and decreased apoptosis. Compared with vector transduction in the control group, improvements in proliferation, cell invasion and glucose transport were observed in the BeWo choriocarcinoma cell line transduced with Ad.IGF-I (45) (46).

Direct intraplacental injection of Ad.IGF-I can correct fetal weight in an FGR mouse model(46). More recent studies in the natural rabbit runt model of FGR found that intraplacental administration of Ad.IGF-I returned musculoskeletal and liver weights to control levels ($P<0.01$), though placental weight remained unaffected. Detection of Ad.IGF-I gene transfer to fetal or maternal organs was

negligible (47). These results are promising, and have the potential for translation into human clinical trials. However, the method of administration needs to be considered, as currently, gene transfer to the fetal side of the placenta is considered excessively risky due to concerns about germline gene transfer and off-targeting effects.

Fibroblast growth factor (C)

Similar to VEGF, fibroblast growth factors (FGFs) are proangiogenic molecules, but they lack the intracytoplasmic sequences that facilitate extracellular transport. However, FGFs are able to bind well to heparan sulphate proteoglycans (HSPGs), which is located within the extracelleluar matrix and on the surface of cells and could be considered an alternative method of cellular delivery instead of the classic cellular transport (48, 49). Its role in angiogenesis has been investigated in the cardiac vasculature of a porcine animal model, where transduction of FGF-5 using an adenoviral vector improved peripheral blood flow following stress-induced myocardial ischaemia. Its relevance in placental insufficiency has not been investigated, but it may have therapeutic benefit in the management of FGR.

RISKS OF PLACENTAL DIRECTED GENE THERAPY (B)

Placental Transmission (C)

The aim of placental gene therapy for FGR would be to reduce or eliminate the adverse effects of poor growth in the fetus by improving maternal vascular perfusion and/or nutrient transport across the placenta, without exposing the fetus to significant amounts of the vector. Transfer of a gene therapy across the placenta to the fetus in significant quantities would be undesirable, as it would risk germline transmission and may have adverse effects on fetal development. Current evidence suggests that the extent of placental transfer depends on the vector, the animal, the route of administration, and the gestation at which it is administered.

In the pre-clinical studies of Ad.VEGF placental gene transfer, local delivery to the uterine arteries has been achieved either by direct injection combined with proximal occlusion of the vessel or by

external application of a thermolabile Pluronic gel to the vessel wall. Maternal intravascular gene therapy using an adenoviral vector gives transgenic protein expression only in the placenta and not in the fetus, when given to pregnant sheep(50, 51), mice (52), and guinea pigs (37), In contrast, adenoviral vector injection into the placenta of rabbits or rats transduces both the placenta and the fetus (53, 54). Exposure of human placental villous explants to high dose adenoviral vector showed that, where the syncytiotrophoblast was deficient there was occasional transduction of the underlying cytotrophoblast, but no evidence of the vector crossing the basement membrane(55). In translation to clinical practice this could be replicated using a balloon catheter, introduced into each uterine artery in turn using x-ray guided interventional radiology. This technique has been used for over 30 years to treat fibroids and manage postpartum haemorrhage. It is now being used increasingly during pregnancy, with catheters and deflated balloons placed into the uterine arteries before Caesarean section when heavy bleeding is anticipated(56).

The pre-clinical studies of Ad.IGF-I have used direct intraplacental injection. This could be easily replicated in the clinical setting under ultrasound guidance, with a similar technique to chorionic villus sampling or amniocentesis. In the majority of pre-clinical studies however, direct intraplacental injection of adenoviral vectors results in transfer to the fetus and the dam (53, 57-59). This is probably because the needle that injects the vector causes a breach in the intact placental barrier.

Germline transmission (C)

Whilst maternal risks regarding germline transmission in pregnancy are no different to those in a non-pregnant woman, vector modification of the fetal germ line could theoretically occur following placental gene therapy. This would be dependent first on whether the vector reached the fetus, and then the germline. The gestation of the fetus is important for germline gene transfer risk. Early gestation direct fetal gene therapy has resulted in germline transmission when retroviral vectors have been injected into the peritoneal cavity of first trimester fetal sheep (60), and after first trimester but not second trimester intraperitoneal injection of lentiviral vectors into fetal macaque monkeys (61). Compared with direct fetal gene therapy, late gestational maternal uterine artery

gene therapy should carry a very low risk of fetal germline transduction. However, this is an important safety concern to evaluate as placental gene therapy translates into clinical application.

Placental toxicity (C)

As there are no published data on the effects of gene therapy on the human placenta *in vivo,* we rely on data derived from pre-clinical studies, which show no evidence of placental toxicity. T cells and macrophages were not markedly increased in placenta of pregnant rabbits treated with an adenoviral vector (62). *Ex vivo* studies assessing the effect of an adenoviral vector containing VEGF in human placental villous explants showed no increases in enzymes pressed by dysfunctional placental cells such as lactate dehydrogenase and human chorionic gonadotropin, when compared with LacZ(55).

ETHICAL AND REGULATORY CONSIDERATIONS FOR CLINICAL TRANSLATION (B)

There are specific ethical and regulatory issues in medical research involving pregnant women. The ethical principles outlined by the World Medical Association's Declaration of Helsinki surmise that clinical trials must be entered into autonomously with consideration of the risk to benefit balance to the patient, with their safety and wellbeing as the predominant concern (63). Whilst the regulation of human gene therapy is essential, the classification of all pregnant women as vulnerable in clinical research in an effort to protect their rights as research participants may actually be obstructing medical advancement (64, 65). Predominant reasoning for excluding pregnant women in research is the uneven prioritisation of fetal wellbeing ahead of the mother, and a consideration that the mother is a vulnerable participant given her pregnancy diagnosis (66). A woman's autonomous participation in a clinical trial may even be questioned if there is a potential therapeutic benefit to the fetus at the expense of the mother (67, 68).

Despite the 2002 Council for International Organizations of Medical Sciences (CIOMS) recommendation that pregnant women should be eligible to participate in clinical studies, progress

in obstetric research has remained slow. In 2009, the United States Code of Federal Regulations continued to classify all pregnant women as vulnerable subjects, though their participation in research was deemed acceptable if data from preclinical studies showed the therapeutic agent having no adverse effects to the mother or fetus(69). Clinical trials in recent years are challenging these exclusions as demonstrated in open myelomeningocele repair and laser ablation for twin-twin transfusion syndrome (67, 70-72). Current obstetric practice routinely employs interventions that are performed with sole benefit to the fetus, for example the use of corticosteroid for neuroprotection and to promote neonatal lung maturation, or caesarean sections for fetal hypoxia.

The EVERREST consortium of academic health science centres, universities and pharmaceutical companies was awarded European Commission funding to investigate the efficacy and safety of Ad.VEGF therapy in pregnant women diagnosed with severe early onset FGR through phase I/IIa clinical trials (73, 74). The consortium were awarded orphan drug status from the European Committee for Orphan Medicinal Products for FGR, the first time that a drug has been recognised for the treatment of FGR(75). In stakeholder and patient interviews conducted by the EVERREST consortium, a primary issue raised when considering a trial of maternal gene therapy for FGR, was the additional psychological distress that is added to an already stressful situation, through asking a pregnant woman to consider taking part in a trial (Table 2). Interestingly, maternal gene therapy was for the majority of stakeholders considered to be acceptable if there was clear fetal benefit (76). Likewise, most women felt they had the capacity to make informed decisions whilst pregnant, which aligns with the recommendations by the CIOMS (68), so long as they were provided with the information required to make an autonomous decision.

Early phase clinical trials in pregnancy (C)

The primary aim of phase I clinical trails is to determine safety. For placental gene therapies where there is no discernable maternal benefit, the ‘Council of Europe Protocol to the Convention of Human Rights’ stipulates clinical trials can only be undertaken when risk is minimal, the research

cannot be transferrable in non-pregnant participants, or where there is immense benefit to future pregnant women with obstetric conditions (77). These directives parallel those stipulated by the United States Code of Federal Regulations Protection of Human Subjects(69). And whilst there should be minimal risk to both the mother and fetus, the risk does should not have to be negligible to enable participation (78).

CONCLUSION (B)

Improvements in vector design and our ever-expanding knowledge on the molecular basis of disease signals a positive future for gene therapy in obstetric conditions. In placenta-directed gene therapy, the results from preclinical studies suggest that viral vectors may be of benefit to target the maternal uteroplacental circulation with low transmission risk to the fetus. The uniquely short time frame with which obstetric research operates offers distinct ethical and practical challenges when conducting clinical trials in humans. Resolving such challenges may improve our knowledge base into the pathophysiology of FGR and how we can medically manage this condition in pregnant women.

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ETHICS (B)

Experimental protocols in animals were performed following the U.K Home Office Regulations and Guidelines for the Operations of Animals (Scientific Procedures) Act (1986) and approved by the local institutional ethics committees.

CONFLICTS OF INTEREST (INCLUDING FUNDING) (B)

Professor Anna David is director and an unpaid consultant for Magnus Growth, a subsidiary of Magnus Life Science, a company that is currently working on a novel treatment for fetal growth restriction.

PRACTICE POINTS (B)

Placental directed gene therapy produces short term expression of a therapeutic transgenic protein and may be useful for treatment of fetal growth restriction

Adenoviral VEGF gene therapy administered via the maternal uterine arteries to the placenta increases fetal growth velocity in pre-clinical studies of FGR.

Placental IGF gene therapy may improve placental transfer of glucose and amino acids and increase fetal growth.

Translating gene therapy for an obstetric disease from the laboratory to the clinic is complex, and requires multidisciplinary expertise

The ethical and social acceptability of using placental directed gene therapy will be influenced by the risks to the mother and the fetus, including the potential for fetal gene transfer.

RESEARCH DIRECTIONS (B)

A phase I/IIa safety/efficacy study on placental directed VEGF maternal gene therapy. Use of other growth factor proteins such as IGF and FGF.

FIGURE LEGENDS (B)

Figure 1: Summary of key findings from two studies of prenatal adenoviral (Ad) vascular endothelial growth factor (VEGF) gene therapy in the overnourished adolescent model of fetal growth restriction (FGR).

In the fetal study, 57 singleton-bearing adolescent dams were offered a control-intake (C) or high-intake (H) diet to generate normal or compromised fetal growth, respectively, and received bilateral uterine artery injections of Ad.VEGF, Ad.LacZ (control vector) or saline at laparotomy in mid-gestation. The fetal abdominal circumference (AC) was measured by ultrasound at weekly intervals

between 83 and 126 days gestation. At delivery at 131 days (0.9) gestation, fetuses were categorized as marked FGR or non-FGR based on a -2SD cut-off relative to the control group mean [B]. In the postnatal study, 33 singleton-bearing overnourished adolescent dams (all H) received either Ad.VEGF or saline and 30 underwent serial measurements of AC between 79 and 113 days gestation [C]. Ewes were allowed to spontaneously deliver near term and lambs were weighed at birth [D]. Exact p values presented show two-group comparisons by t tests. *** $p<0.001$ and * $p<0.05$ indicate levels of significance for overall ANOVA. Post-hoc comparisons are indicated by capital letters. A and B show time points at which AC measurements were significantly greater in H+Ad.VEGF versus H+Saline / H+Ad.LacZ groups ($p=0.016–0.047$).

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