Project title	**Pregnancy complications: targeted interventions**		
Key words	Pregnancy, mouse, placenta, therapeutics		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research	Yes	
	Regulatory use and routine production		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals	Yes	
Objectives of the project	Complications of pregnancy affect around 1 in 6 pregnancies in the UK and cause enormous social and financial burden. Some of the most common pregnancy complications include Fetal growth restriction, preeclampsia and gestational diabetes associated with fetal overgrowth. Fetal growth restriction (FGR), relates to the inability of a baby to achieve its genetic growth potential. FGR significantly increases the risk of stillbirth and also leads to a greater risk of adulthood diseases such as heart disease. Preeclampsia (PE) is associated with increased maternal blood pressure and the presence of proteins in the urine, indicating sub-optimal kidney function. In addition, PE is associated with an increased risk of having an FGR baby making PE a high-risk pregnancy for mother and baby. Gestational diabetes mellitus (GDM) is characterised by excess glucose in the blood which arises during pregnancy and puts the baby at greater risk of being overgrown. An overgrown baby increases the risks of complications during delivery and, in addition, a baby that is overgrown at birth is at greater risk of obesity and diabetes in adulthood. Thus, complications of pregnancy have implications that can last a lifetime. Despite these devastating consequences, there are no treatments for FGR/PE other than early delivery of the baby which is itself associated with poor outcome. One of the reasons for this lack of therapeutics is that we do not fully understand the		

	mechanisms underpinning these complications of pregnancy. It does appear that abnormal placental function is key to the onset of these complications but many of the exact mechanisms remain elusive. As such, it remains imperative that we continue to assess the changes in placental function that accompany these complications and to target therapeutics based on this evidence. As such, we have already demonstrated, in mouse models of FGR, that sildenafil citrate (Viagra) is one drug that may have therapeutic value in the treatment of FGR. We have shown that Viagra improves placental blood flow (which is often impaired in FGR) and increases fetal growth as a result. Following these data, a human clinical trial has been funded emphasising the potential for mouse models to provide a good pre-clinical testing ground. This project has 3 major objectives: 1. To identify whether signals derived from the fetus are important in the control of fetal growth and whether these signals are different/absent in cases of poor fetal growth 2. To identify new potential therapies for FGR/PE/ fetal overgrowth by focussing on both dietary modifications and drugs already approved in the clinic for other diseases that share similarities to pregnancy complications (e.g. those designed to increase blood flow) 3. To target these therapies specifically to the placenta, both to minimise possible side effects of therapies and also to maximise the chances of success of these therapies. Targeting involves attaching a protein 'tag' to these drugs which allows them to bind only to the placenta, maximising action of these drugs at the required site. This should minimise the risk of possible side-effects caused by drugs being delivered to multiple organs.
Potential benefits likely to derive from this project	Following this project, there will be a greater understanding of the placental mechanisms that underpin FGR, PE and the fetal overgrowth associated with GDM. As part of this insight, we will have a greater idea as to whether signals from the fetus to the placenta are important in fetal growth and whether these signals are altered/absent in complications of pregnancy. This project also has the potential to identify further candidate therapeutics, in addition to Viagra. These candidate therapeutics may include dietary modifications such as beetroot juice, which contains constituents (nitrate) shown

	to improve blood flow in non-pregnant humans and animals. In addition, we will have evidence as to whether drugs targeted to the placenta only give additional benefit in terms of safety for mum and baby, and in terms of achieving greater therapeutic value compared with the same drugs delivered to the entire body? In terms of the potential benefits, results from this study will be of use to other animals, since animals such as pigs, cats and dogs often have large litter sizes with resultant runts that are at risk of death during pregnancy or after birth. As such, any potential therapies suggested for the treatment of FGR following this project have the potential to be used in veterinary medicine.
Species and approximate numbers of animals expected to be used, and anticipated period of time	All experiments will be conducted in mice. Approximately 4000 will be used during the 5 years duration.
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	In terms of the administration of potential therapeutics, most of these will be administered via the diet (water or food). For substances that are unable to be administered in this manner, this will be via an injection, either under the skin or into the abdomen. This will cause a mild and transient pain. For experiments when surgery will be required (e.g. insertion of blood pressure probes, perfusion of umbilical blood vessels), the animals will be kept at a surgical plane of anaesthesia so as not to feel pain and surgery carried out using sterile techniques to minimise risk of infection. When animals have recovered from surgical anaesthesia, animals will be monitored for signs of pain and/or distress. In surgeries that involve suturing, there is a small risk that animals may remove sutures. If this occurs, and the incision site appears clean and infection-free, the animal will be re-anaesthetised and re-sutured up to a maximum of 1 additional anaesthetic exposure. In the unlikely event that the wound becomes infected, causing pain to the animal, animals will be humanely euthanized. Additionally, animals will be given painkillers as required post-surgery but, when animals appear in significant pain or distress, we will seek veterinary advice and humanely euthanize as required., For most of our procedures, particularly the dosing regimens chosen, we do not expect animals to suffer any lasting pain or distress.

	Some animals will undergo blood pressure measurements which involves restraint in a tube and cuff inflation around the tail of the mouse. This causes transient discomfort/stress but those animals shown to be overly stressed (e.g. as evidenced by rapid heart rate) will be removed from the tube. At the end of each protocol, all animals will be humanely euthanized by a Home-Office approved method.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	One of the reasons why so little progress has been made in developing drugs for pregnancy diseases is that clinical trials testing treatments in pregnant women are very difficult. For example a drug targeted to the alleviation of mother's symptoms could cross the placenta and prove detrimental to the health of the baby. Thus, in order to assess the effectiveness of potential therapeutics, the use of animals is the only possible starting point. We always run experiments using human placenta in the laboratory alongside animal experiments as a first step in determining effectiveness in women but such experiments cannot inform us of any general beneficial or harmful effects to mother and fetus or their function when a blood supply is intact. Computer modelling of the pregnant woman is just not possible with our present state of knowledge.
2. Reduction How the use of minimum numbers of animals will be assured	We will keep the number of animals to a minimum by making as many observations/measurements as possible on individual mice (also aided by the fact that each litter comprises multiple pups) and by removing as many tissues as appropriate for later analyses. This ensures that from one pregnant mouse, we can obtain multiple datasets. As we have several years experience of similar experiments on mice we can be confident of the minimum numbers we will need to achieve statistical significance. Experiments will be designed so that the primary statistical test will be to assess 2 variables, treatment (treated v untreated) and genotype (genetically altered versus wild-type controls).
3. Refinement Reasons for the choice of species and why the animal	As we require knowledge on placental function, a mammalian species is essential for our work. The mouse has a uterus and placenta similar to that in women and also allows us to study genetically modified strains that

model(s) to be used are the most refined, having regard to the objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	have disease symptoms similar to those found in humans. Such accurate disease models are not available in any other species. We will minimise suffering by using anaesthetic for any potentially painful procedures and by careful monitoring of the animals to ensure they are not in discomfort. For recovery surgery procedures, analgesics will be used as necessary to minimise pain. Additionally, for administration of therapeutics, this will occur primarily via drinking water or in the food. Only if this is not possible, will injections or insertion of minipumps underneath the skin be employed. Whilst we do not expect any adverse reactions from our candidate therapeutics, the use of targeted treatments will further limit any off-target effects.