Project title	**The long-term effects of prenatal hypoxia on cardiomyocyte function**		
Key words	Programming, hypoxia, cardiac, mitochondria, myocyte		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research		No
	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		No
Objectives of the project	The overall objective is to assess the long-term effects of prenatal hypoxia on cardiomyocyte function. This goal will be realized by addressing the following specific objectives in fetal, juvenile and adult mice previously exposed to prenatal hypoxia: 1. To measure in vitro cardiomyocyte contractility and Ca2+ homeostasis 2. To assess In vitro cardiomyocyte mitochondrial function 3. To characterize gene expression and modification of key cardiomyocyte proteins		
Potential benefits likely to derive from this project	The present project will investigate the long-term effects of prenatal hypoxia on cellular function of the heart. The main benefit of the study is the advancement of current understanding of the cellular and molecular mechanisms underlying cardiac programming in response to prenatal hypoxia. We hope to identify cellular targets for drug intervention to protect people from developing cardiovascular diseases later in life. All of the findings will be published in peer reviewed leading scientific and clinical journals as appropriate to ensure wide dissemination of the research findings. The information is of direct benefit to basic scientists, physiologists and		

	clinical cardiologists and will provide key information enabling better management of cardiovascular disease.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Wildtype mice, approximately 850 animals over 5 years
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	Adverse effects: 1. Maternal reduced food intake, activity and weight: a. Severity band, mild b. Dams exposed to hypoxic environments are known to exhibit a decrease in food intake (up to 40%) and a substantial decrease in physical activity, leading to a decrease (~20%) in maternal body weight. 2. Maternal preeclampsia-like symptoms: a. Severity band, moderate b. Hypoxia during pregnancy can cause maternal preeclampsia-like symptoms such as hypertension, proteinuria and kidney pathology. 3. Intrauterine growth retardation (IUGR) and physiological and morphological defects associated with prenatal hypoxia a. Severity band: Moderate. b. Prenatal hypoxia causes IUGR and a host of physiological and morphological defects, some of which persist into adulthood. 4. Disease susceptibility in offspring. a. Severity band: Moderate. b. Although we are not specifically inducing this, it is possible that offspring exposed to prenatal hypoxia will experience disease susceptibility in association with aging (i.e. cardiovascular diseases, such as heart failure) later in life. All animals will be sacrificed according to Schedule 1 at the end of the protocols
Application of the 3 Rs	
1. Replacement Why do animals	Adult cardiac myocytes are terminally differentiated and cannot be maintained in tissue culture conditions as they

need to be used, and why non-animal alternatives cannot be used.	de-differentiate and alter their function. There are no suitable cell lines that can be used to fill these purposes as those that are used (embryonic myocytes and the HL-1 cell line) are derived respectively from embryonic tissue and an atrial tumour line. These cell lines are morphologically and functionally quite different to adult cardiac myocytes. Moreover, we will be studying the long-term effects of prenatal hypoxia on cardiomyocyte function, which cannot be reproduced using cell culture techniques nor can they be suitably modelled using computer simulations given the lack of understanding of the fundamental processes. The use of human tissue is not possible due to several reasons: 1) of limited availability, 2) rarely not already diseased and 3) nearly always subject to pharmacological interventions. These factors again limit the utility of human resources when elucidating basic fundamental mechanisms.
2. Reduction How the use of minimum numbers of animals will be assured	Experimental design has been discussed with, and approved by, a statistical advisor. In order to minimise the number of animals required, sample size has been estimated for each experiment based on existing published data and the use of power analysis (desired power of 0.8, $\alpha = 0.05$). These estimates will be updated and recalculated throughout the project as we generate new data.
3. Refinement Reasons for the choice of species and why the animal 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.	We are committed to using the most translationally relevant model. We have chosen the mouse as our experimental species for several important reasons: 1. Mice have a short generation time and an accelerated lifespan (2 years) which allows the long-term effects of prenatal hypoxia to be studied within a reasonable timeframe. 2. Our ability to directly manipulate the mouse genome provides an incredibly powerful tool to identify and confirm molecular targets for drug intervention. 3. Due to their small size and short generation time, maintaining mice requires less resources and space, and the time required to perform research is manageable. 4. The mouse has large litter sizes which allows the generation of multiple, identically reared progeny. Steps to minimise welfare costs to animals: 1. Basic requirements for good rodent housing and

	husbandry will used at all times. 2. It is not possible to house pregnant mice in groups, but once pups have been weaned, mice will be housed in stable, compatible groups. 3. The following parameters will be measured during the protocol to ensure animals remain within the outlined severity limits: Body weight, body condition scoring (BCS), food and water intake and cardiovascular status. Control animals not subjected to any procedures will be used as a benchmark for normal changes in these parameters. 4. Oxygen levels will never be reduced lower than 9% 5. When animals are first put into the chamber, oxygen levels will be normal (21%) for 24 hours and then reduced slowly (over another 24 hour period) to avoid shock.