Home Office

NON-TECHNICAL SUMMARY

Breeding and Maintenance of Gal-deficient Swine

Project duration

Years **5**
Months **0**

Project purpose

- (a) Basic research

Key words

No answer provided.

Animal types	Life stages
Pigs	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Animal tissue commonly produces high levels of a carbohydrate antigen called Gal (galactose alpha 1,3 galactose) which is not present in humans. Since humans do not make this carbohydrate they produce anti-Gal antibody, much like occurs in the case of blood group antigens. The research group has shown that Gal is present in commercial porcine and bovine derived biological heart valves (BHVs) and that human antibody binding to Gal accelerates tissue calcification. Antibody induced calcification does not occur using Gal-free tissue from GT-knockout pigs (GTKO). This project is designed to maintain a breeding colony of GTKO pigs to produce tissues for *in vitro* research which aims to establish the basic physical and biological equivalence of GTKO compared to commercial porcine and bovine derived tissue. This will lead to a more fundamental understanding of the process of tissue calcification and the role of antibody in that process. The Gal-deficient GTKO pigs represent a unique resource which forms the core technology needed for the development of Gal-free bioprosthetic devices.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The primary benefit of this PPL is that it provides tissues and materials to researchers developing new therapies for cardiovascular disease. These new technologies are trying to improve the performance of replacement biological heart valves (BHVs), especially in patients under 60 years of age. This is experimental work but if successful the new valves will broaden the available therapies to treat younger patients, giving them a durable device which will not require lifetime anticoagulation medication and thereby avoids the serious thrombo-embolic risks associated with anticoagulation. This would have a major impact in developing nations with endemic levels of rheumatic fever a major cause of heart valve dysfunction, where the resources to manage patients on anticoagulation are limited and therefore the treatment of young patients may not be optimal or in many cases is not available at all.

What outputs do you think you will see at the end of this project?

This project will supply us with GTKO pericardial tissue to support the production of GTKO pericardial biological heart valves for use in ongoing and future *in vivo* studies in juvenile sheep. These studies are designed to compare the biological equivalence of GTKO pericardial biological heart valves to pericardial biological heart valves made with normal (wild-type) tissue. This is necessary to demonstrate to that the mutation in the alpha-galactosyltransferase gene does not compromise the GTKO pericardium in some unexpected way.

This project will also supply tissue for *in vitro* durability testing of our GTKO pericardial biological heart valves and future *in vivo* comparison in juvenile sheep of our GTKO pericardial biological heart valve with commercially available biological heart valves.

If all of these tests are successful, we will be poised to begin producing our GTKO pericardial heart valve under Good Manufacturing Conditions (GMP) with the aim of gaining regulatory approval to start human clinical trials.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

This is an experimental program developing a new GTKO heart valve. If the program is successful, showing physical and biological equivalence of wild type and GTKO tissues/valves, high GTKO valve durability (greater than 200 million cycles) and equal in vivo performance compared to existing commercial devices, we will be positioned to begin GMP production of this heart valve which might then be used in a first in man trial.

These valves, because of their lack of the Gal antigen, are expected to resist anti-Gal antibody induced calcification and have improved durability in all age groups but would be most beneficial to younger patients

How will you maximise the outputs of your work?

The results of this work will be published in high quality peer reviewed journals.

Species and numbers of animals expected to be used

- Pigs: 100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Genetically altered pigs with no expected harmful phenotype will be bred under this project. Each animal will be genotyped following collection of DNA by mouth swabbing or blood sampling. Selected pigs will be maintained to maturity for breeding purposes.

All animals are reared to at least 180 day and humanely killed for tissue harvest.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Pigs bred under this protocol are not expected to show any harmful observable difference in appearance, development, and/or behavior due to their genetic modification.

All pigs will be housed and reared and bred as per standard practices for a normal pig colony.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Mild 100%

What will happen to the animals at the end of the study?

- Killed

Application of the three Rs

1. Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Tissue samples obtained from Gal-deficient GTKO pigs are required for laboratory purposes to understand the impacts of using Gal-deficient porcine materials in bioprosthetic devices and to support development of a new GTKO pericardial biological heart valves.

What was your strategy for searching for non-animal alternatives?

There are no other alternatives.

Why were they not suitable?

N/A

2. Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers will be adapted as necessary to satisfy the tissue quantity required for bio-prosthetic heart valve manufacture, and/or biological testing. The estimated animal number is based on the predicted amount of tissue required over the next 5 years.

Typically a breeding colony of 5-6 is maintained, increasing to a maximum of 15 if tissue demand increases.

Animals will only be mated to maintain the health of the line and to satisfy the requirements for provision of basic materials for in vitro analysis.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Animals will only be mated to maintain the health of the breeding stock and to satisfy the requirements for provision of basic materials for in vitro analysis. Care will be taken to ensure the minimum number of animals is produced.

What other measures apart from good experimental design will you use to minimise numbers?

The scale of breeding is matched to the requirements for tissue and maintaining at the minimum breeding stock to maintain the health of the colony (typically 5 animals).

The few excess GTKO animals not used for tissue donation are used to replace breeding stock.

The animals used in this project are used for non-invasive training and teaching purposes and tissues from wild-type animals are available to other groups under our cadaver sharing policy.

3. Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Standard porcine husbandry practices are used.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The material demands for producing replacement heart valves (e.g. the necessary size of the tissue) requires us to use an animal of substantial size. This would exclude smaller mammals, mice or rats, with the GGTA-1 mutation. Swine currently are the source of many biomedical materials and are appropriately sized matched for humans. Bovine strains with GGTA-1 mutations have been reported and bovine pericardium is widely used to make bioprosthetic heart valves. Due to their large body size and small litter size cows require far greater resources and time for genetic modification compared to pig and would likely be a more expensive source of GTKO materials for our work. There is no experience using tissues from non-mammalian sources (birds, fish, or reptiles) for making BHVs and these animals, while less sentient, would likely be wild caught, in limited supply and maybe under protected status.

GTKO tissue is typically sourced from pigs over 180 days old to ensure enough tissue is available to produce the bioprosthetic devices.

What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Animal staff monitor pigs throughout the night at farrowing to ensure any complications are detected early and where necessary assistance provided according to standard veterinary practice.

Freedom farrowing crates, which allow the sow a greater degree of movement during farrowing and prior to weaning are being tested as an alternative to standard farrowing crates. Pigs are weaned at 4 weeks to minimise the time that the sow is restricted in movement, whilst ensuring the welfare of the piglets.

Buccal swabs are collected as the most refined way to genotype the pigs, this is a refinement that has been implemented over the previous PPL to replace tail tipping. Blood sampling remains as a back-up as buccal swabbing larger pigs can be stressful as restraint is more difficult. In the previous PPL only buccal swabbing was actually used.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Home Office Code of Practice

DEFRA Welfare Codes for Pigs

How will you ensure you continue to use the most refined methods during the lifetime of this project?

NC3R website will be used as a source of information of advances in 3Rs, as well as review of the regular updates received from the designated establishment. Any advance considered appropriate in this PPL will be incorporated where possible.

Explain the choice of species and the related life stages

Tissue samples obtained from Gal-deficient GTKO pigs are required for laboratory purposes to understand the impact of using Gal-deficient porcine materials in bio-prosthetic devices and to support development of a new GTKO pericardial biological heart valve.

Tissues are harvested post-mortem from pigs, greater than 180 days of age, to provide sufficient pericardial material for valve manufacture.

Adult pigs will be used for breeding purposes to maintain the colony and supply of tissue.