Private & confidential: *Please be aware that the contents of this form may be made public resulting from the "Freedom of information Act". Personal details will not be released.*

G: NON-TECHNICAL SUMMARY (NTS)

Please attach the Non-technical Summary as generated by your application in ASPeL.

Project Title	Maternal fasting and offspring health
Key Words	Ramadan, fasting, pregnancy, cognition
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Healthy adult Muslims are required to fast during Ramadan. Pregnant women are exempt; however the majority take part in the daily fast. The impacts of intermittent fasting on the health of the developing fetus and adult offspring are not fully known. A poor or imbalanced diet during pregnancy is known to slow the growth of the fetus and lead to both cardiovascular disease and poor mental health. The aim of the proposed study is to determine whether intermittent fasting in pregnant rats, which models aspects of human fasting during Ramadan, has detrimental effects on growth of the fetus and the cardiovascular and mental health of the offspring. We will also study the role that the bacteria in the intestine play in the development of the brain and whether the mother's diet during pregnancy affects the signals that the bacteria send to the offspring's developing brain.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Worldwide there are 230 million Muslim women of childbearing age. On average, Muslim women have 3 children. Some women will be pregnant outside Ramadan, but up to ¾ of all pregnancies will coincide with Ramadan at some stage. Therefore there are potentially 517 million babies globally that have been exposed to intermittent fasting before they were born. Studies in Muslim countries suggest that the incidence of mental impairment is greater in children whose mothers fasted while pregnant. Unlike other forms of undernutrition during pregnancy, which are usually driven by poverty, fasting during Ramadan is voluntary. Many Muslim women seek assurance from health practitioners that fasting during pregnancy will not be harmful to their baby; however the advice available to pregnant women is contradictory. Clear guidance upon which women can base an informed choice is lacking; therefore there is a need to understand what happens to the fetus if the mother chooses to fast while pregnant.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use rats as an experimental model for this project which will last five years. We estimate that we will use 1550 animals.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Experiments will start by removing food from pregnant rats overnight, which is when they are active and would normally eat most food. In some cases this will be for a few days of pregnancy; in others it may be for the majority of pregnancy. The animals will feel hungry, but they will have the opportunity to eat as much as they wish when food is returned each morning. In some experiments we will remove water overnight, in others we will remove both food and water in order to mimic the fasting that humans voluntarily undertake during

Ramadan. To study the movement of nutrients across the placenta from the mother to the fetus we will use non-recovery anaesthesia so the animals will not suffer any pain. In other experiments we will let the mothers deliver their litters normally. Some of these animals will be used to study memory, learning and behaviour. Sometimes food will be removed overnight prior to the test so that food can be used as a reward to motivate the animals. In the majority of tests the animal's environment will be enriched to stimulate it to explore or interact with others; therefore there will be no adverse effects. Experiments designed to look at the bacterial content of the intestines will use samples of faeces passed normally by the rats or tissue collected from animals that have been killed by an approved method. The final phase of the study will involve feeding rats either a high salt or high fat diet after they have been weaned. Rats eat such diets readily, although the diets will lead to an increase in blood pressure and the risk of diabetes. Blood pressure will be measured using a device applied to the animal's tail which is similar to a human blood pressure cuff. To do so, the animal must be restrained which causes some distress; however this is minimised by training. Urine will be collected by placing the rats in cages with wire grid floors: they find such cages distressing so we will minimise harm by holding them for as short a time as possible to collect enough urine for analysis (usually 2-4 hours). In order to test for diabetes we will use a glucose tolerance test which involves injecting a glucose solution into the abdomen and then collecting small quantities of blood from the tail repeatedly over the following 2 hours. This will cause brief discomfort when a needle is inserted. The final experiments will involve a study of kidney function; however as this is done under non-recovery anaesthesia it will not cause any pain or distress to the animal.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We will be able to use human placentas for some of our planned studies; however it is not possible for ethical and safety reasons to involve pregnant women or their children in some of the more invasive experiments designed to help us understand underlying biological mechanisms. We must therefore use an animal species that has a placenta for the work outlined in this licence. As only mammals have placentas, we cannot use a lower species; therefore we have chosen to use rats.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have designed our experiments carefully so that we can get as much information as possible from each animal. For example, an animal that has been used for behavioural

experiments can also go onto provide tissues for follow up molecular experiments. We have based our estimates of animal numbers on our own experience with the model, so we have a realistic understanding of how many rats will be required to complete each experiment.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

We have chosen to use rats as they have large litters (usually around 15 pups) so we can get as much information as possible from one pregnancy. Their size makes them preferable to mice as they are better suited to the planned surgical procedures; hence the success rate will be greater. In our earlier studies, we fasted pregnant rats daily for the whole of pregnancy. However, in order to more closely mimic the duration of human fasting during Ramadan we have refined our model to fast animals for 3 days, which is equivalent to 1 month of a human pregnancy.