

NON-TECHNICAL SUMMARY (NTS)

Project Title	Identification of critical factor(s) required for optimised embryo development
Key Words	sheep, biomarker, embryo transfer, hyaluronan
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;
Yes	(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):

Poor fertility is a major problem in humans and domestic animals representing a significant economic loss for farmers and social discomfort to the couple. Currently 1 in 7 couples seek assisted reproduction technology (ART), with failure to implant a common reason for infertility. Similarly, in domestic animals, failure to produce offspring in a regular interval is a limitation to animal production and is one of the main issues for dairy cows.

The establishment of pregnancy needs both the presence of a good quality embryo and a womb at the correct stage to receive it. To date, embryos produced in the laboratory develop better if transferred to the oviduct where the embryo will form after natural mating and spend early stages before transfer to the uterus for implantation. This shows that culture conditions are deficient and further scientific research is required to improve formulation of embryo culture media.

Using funds from BBSRC, we have shown that a molecule called hyaluronan (HA) which is present in most mammalian tissues has an important role in embryo development. It is produced in different sizes in the reproductive system and by the embryos at later stages of development (blastocyst stage). We have also shown the presence of Hyaluronidase (Hyal-2) (an enzyme which breaks HA into smaller fragments) in the oviduct. The small HA fragments can function as a survival factor and we have shown that they improve cow and sheep embryos quality and support their development to a later stage. Similar beneficial effects were observed when this enzyme was infused into the sheep oviduct. Enzymes with similar biological functions are delivered by spermatozoa to the reproductive organ during natural mating and are present at the fertilisation site in the oviduct. We believe that these enzymes or their end products have the potential of being used as supplements to commercial embryo culture media.

We have designed experiments to test the effects of different Hyals on development and quality of sheep embryos produced in our laboratory from abattoir-derived ovaries. In addition, we will transfer the blastocysts to live recipient ewes to assess improvements in pregnancy rate after embryo transfer. In addition, we will be using human embryos donated to the research to investigate impact of Hyals and their possible use in human ART. This particular work does not involve animals and is not regulated under ASPA.

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)?

The unique feature of this project is the collaboration of an animal embryologist and a human assisted reproduction technologist. The main benefit of this project will be for improving efficiency of assisted reproduction technology both in human and animals including endangered animal species. The quality of embryos is a critical factor in determining pregnancy outcome after embryo transfer. We expect to find that hyaluronidases improve embryo quality and result in higher pregnancy rate. This project will also investigate mechanism of action of hyaluronidases. Therefore, scientific community and clinician active both in animals and human will benefit from the data generated.

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

95 sexually mature female sheep

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?

Surgical embryo transfer is a routine method in sheep breeding. The adverse effects are not specific to this procedure. Similar to any surgical procedure, there will be possibilities of fatality during anaesthesia or due to post-operative infection. Maximum care will be taken to work under absolute aseptic conditions. No fatalities occurred in animals which underwent a similar procedure carried out previously by the project leader. The pregnant animals will be kept till term to assess health and normal function of the offspring confirm intact. The lambs will be kept to reach sexual maturity and allow natural mating. Pregnancy rate of the lambs will be assessed on day 35 by ultrasonography. All the animals including pregnant lambs and non pregnant ewes will be returned to the flock (subject to certain restrictions).

Application of the 3Rs

Replacement

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

Replacement

Current *in vitro* techniques are only able to predict developmental potential of embryos. In order to determine quality of embryos and their ability in establishment of pregnancy, it is essential to transfer the *in vitro* produced embryos to recipient animals.

Reduction

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

Reduction

The number of animals requested for the experiment was calculated using *in vitro* data and a statistical programme. In parallel to *in vivo* experiments, we will also carry out *in vitro* experiments using sheep uteri and ovaries collected from an abattoir.

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

For the experiments described in this project, sheep are the preferred experimental animal as they are one of the most similar animal models to human embryo development, so will provide more accurate results.

Maximum care will be taken for the welfare and reducing suffering distress and pain of the animals. One cause of the animals suffering is using inappropriate method of handling during experimentation.

Other cause of the animals suffering will be during administration of substances for injection of drug before or after surgery or during blood sampling. This will be minimised by using a needle gauge appropriate for sheep and the route of injection. Dose volumes will be kept to the minimum required to obtain results and within safe volumes. The surgery procedure will be performed under general anaesthesia. The animals will receive pre-emptive and post-operative analgesia to minimise discomfort and suffering.