CRACK IT

EASE: EliminAting Surgical Embryo transfer in mice

Background

Genetically altered (GA) mice are used extensively to study the function and regulation of genes and their role in human development and disease. In 2015, approximately 50% of the animals used for scientific procedures in the UK were for the creation and breeding of genetically modified animals, the majority of which are mice (Home Office, 2015).

The generation of GA mouse models involves one of three techniques that use embryos at different stages of development:

- Pronuclear injections take advantage of one-cell embryos, where DNA or RNA is injected directly into the pronucleus of a fertilised egg. This technique allows scientists to use gene editing techniques like CRISPR/Cas9.
- *In vitro* fertilisation (IVF) is used to produce two cell embryos. This is a particularly useful technique because it allows scientists to archive or exchange mouse strains as sperm when it is appropriate to do so.
- Embryonic stem (ES) cell injection takes advantage of ES cell culture techniques that allow complex gene targeting events to be performed *in vitro*. Quality Control (QC) verified ES cells are injected into blastocysts (3.5 day old embryos).

Embryos from all three approaches are transferred into pseudopregnant recipients. The majority of embryo transfer procedures performed in mice use surgical techniques, with implantation into the oviduct or uterus via a laparotomy performed under general anaesthesia (Nagy *et al*., 2014). Although this technique is well understood and relatively high implantation rates can be achieved, it is an invasive procedure and can lead to discomfort and surgical complications. In recent years, efficient Non-Surgical Embryo Transfer (NSET) techniques have been developed (Steele *et al*., 2013; Cui *et al.,* 2014) and specific transfer devices are now commercially available.

The NSET approaches pass a catheter through the cervix, allowing embryos to be deposited directly in the uterine cavity. No surgery is involved, offering significant welfare gains. In competent hands these devices work as well as surgical embryo transfer techniques. However, the NSET techniques can only be used to transfer late stage pre-implantation embryos (i.e. the blastocyst stage (E3.5)). This makes the technique suitable for transferring embryos after ES cell injection but it is not suitable for earlier embryonic stages including one and two-cell embryos generated from IVF and pronuclear injection programmes which still need to be transferred into the oviduct using surgical techniques.

Past experience has shown that extended culture of one and two-cell embryos generated from IVF programmes or following pronuclear injection severely compromises their implantation success. This means that these common techniques do not get used in conjunction with the current NSET systems. However, optimising embryo culture conditions is an area of intense research in both the mouse and clinical field. For example, a recent publication by Truong *et al.,* has reported that adding antioxidants (e.g. acetyl-L-carnitine, N-acetyl-Lcysteine and α-lipoic acid) increases blastomere numbers in *in vitro* cultured embryos, as well as improving fetal development with increased crown-rump lengths and fetal weights. These data indicate that altering the redox potential of embryo culture systems may be advantageous when culturing embryos, prior to NSET. Other work has also shown that the addition of myo-inositol, a precursor of phosphoinositides, also significantly increases the number of blastomeres in embryos that had been produced by a microinjection technique called intracytoplasmic sperm injection (Colazingari *et al., 2014).*

NC 3Rs

The aim of this CRACK IT Challenge is to maximise the use of the NSET technique either by the development of a reliable system for culturing *in vitro* manipulated embryos through to the blastocyst stage or the development of an approach to non-invasively manipulate the uterine environment.

3Rs benefits

Developing a robust system that combines an improvement in *in vitro* embryo culture conditions with the use of contemporary NSET techniques would eliminate the need to use surgical embryo transfer when implanting one and two-cell embryos. This would have significant benefits to all of those involved in the development of GA mouse models in terms of improved animal welfare and reduced costs.

Removing the need for surgical embryo transfer would eliminate a significant number of invasive procedures. Based on a survey completed by the International Society for Transgenic Technologies between September 2008 and September 2009 (Fielder *et al.,* 2010), it is estimated that in excess of 250,000 surgical embryos transfers are currently performed globally each year. The MRC's Mary Lyon Centre performs in excess of 2,500 surgical embryo transfer procedures per year. With a viable alternative to surgical transfer of one and two-cell embryos, up to 90% of all invasive embryo transfer procedures could be substituted with a more refined technique.

The availability of techniques developed through this Challenge would also benefit other ongoing research efforts. For example, in recent years the GA mouse breeding community has made huge improvements in the way it exchanges (Kenyon *et al.,* 2014) and stores mouse strains in repositories such as the European Mouse Mutant Archive (Guan *et al.,* 2014). In both cases the preferred option is to use frozen sperm which needs to be recovered using IVF techniques to produce two-cell embryos.

The advent of new gene editing techniques like CRISPR/Cas9 also means that many transgenic laboratories have switched their activities away from ES cell/blastocyst injection and are now concentrating on one-cell injection techniques. This has the potential to increase the number of surgical embryo transfers performed unless NSET techniques can be optimised.

Overall aim

The overall aim of the EASE CRACK IT Challenge is to generate an approach that improves the implantation rates of early stage embryos when combined with extended *in vitro* culture and non-surgical embryo transfer techniques. Using surgical procedures, implantation rates for unmanipulated two-cell embryos are currently ~40%. However, micromanipulated embryos are less robust with ~25% currently giving rise to live offspring.

Need for collaboration

To solve this Challenge expertise in the following areas is required:

- Expertise in embryo culture systems to improve implantation rates of *in vitro* cultured embryos. It will be necessary to understand the complex array of nutrients required for robust *in vitro* embryo development and to determine reliable methods for assaying the success of these culture conditions. It may also be necessary to investigate the effects of different oxygen tensions within the culture conditions.

- Expertise in murine physiology/anatomy to determine:

    - Why uterine transfers do not result in pregnancy when one or two-cell embryos are transferred directly into the uterus.

    - How manipulation of the uterine environment could overcome the barriers to implantation.

Key deliverables

Development of a procedure that improves mouse embryo implantation rates when *in vitro* manipulated, one and two-cell embryos are used in conjunction with NSET. Comparable rates of implantation between NSET and surgical techniques should be achieved. Various approaches may be investigated, for example:

- Improving *in vitro* culture conditions of one and two-cell embryos grown to the blastocyst stage either by:
    - Manipulating the uterine environment so that one or two-cell stage embryos can be transferred directly into the uterus, prior to successful implantation.
    - Creation of a culture system or uterine environment that supports *in vitro* embryo development from the one-cell stage (E0.5) through to blastocysts (E3.5)- a stage that is permissive for NSET. At the present time, *in vitro* culture systems do permit embryo development, frequently achieving 100% development to blastocysts but *in vivo* implantation rates are compromised.
- The technology should deliver implantation rates (that is pups born/embryos transferred) that are comparable with those of current surgical procedures:
    - Implantation rates of ≥40% when using unmanipulated IVF embryos
    - Implantation rates of ≥25% when using microinjected embryos.
    - The solution should be applicable to a high throughput service laboratory where the emphasis is on service delivery, rather than on academic enquiry.
    - The solution may involve the use of additional equipment but it needs to integrate easily into an existing laboratory environment and should not involve any additional procedures that may cause harm to the mice.
    - The product should offer value for money.
    - The solution may involve a combination of improved embryo culture conditions, plus modifications to the uterine environment but it should not be technically challenging for the end-user.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

The Sponsor will provide:

- Expertise in cryopreservation, distribution, breeding and mouse genetics.
- Expertise in mouse surgery and improvements in blastocyst (*in vivo* derived only) implantation rates using NSET devices.

- In-house, *in vitro* and *in vivo* validation work to test the success of the approaches under development. This will include a comparison of surgical and non-surgical embryo transfer success.

- Specific advice and guidance on requirements.

- Introductions to other potential end-users.

- Engagement with the clinical and mouse communities with the outcomes of this Challenge should it offer an improvement to the current *in vitro* culture systems and/or provide opportunity for successful manipulation of the uterine environment.

Duration

Up to one year.

Budget

Up to £100k

Sponsors

The MRC Harwell Institute

References

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