EASE: EliminAting Surgical Embryo transfer in mice

Sponsors: Mary Lyon Centre – MRC Harwell

Launch Meeting

08 September 2016
The Mary Lyon Centre (MLC)

- Located at the MRC Harwell Institute
- An integrated campus for mouse genetics

Mary Lyon Centre

Mammalian Genetics Unit

‘Advancing medicine and knowledge through the discovery and investigation of mouse models of human disease’
MLC service portfolio

Molecular and Cellular Biology → Generation of new GA lines and archiving → Breeding and Colony Management → Phenotyping and in vivo experiments → Ex-vivo testing and Pathology

Gene or Phenotype → Translational studies

Project management

CRACK IT
The Challenge

‘To generate a procedure that improves implantation rates of mouse embryos when combined with extended \textit{in vitro} culture and non-surgical embryo transfer’

Highly invasive

Refined procedure
Why was this Challenge developed? Background

- Need to generate/preserve GA mice
- 3 principle approaches:
  - Pronuclear injection involving DNA/RNA injection into the 1-cell embryo
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- **3 principle approaches:**
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  - ES cell injection allows complex gene targeting events to be performed
  - IVF/ET is used when re-establishing/exchanging existing mouse strains
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- 3 principle approaches:
  - Pronuclear injection involving DNA/RNA injection into the 1-cell embryo
  - ES cell injection allows complex gene targeting events to be performed
  - IVF/ET is used when re-establishing/exchanging existing mouse strains
- Surgical embryo transfer is the technique of choice in most cases
Why was this Challenge developed? Context

- Surgical ET used for E0.5 to E3.5 embryos
  - ~40% implantation rates
- Non-surgical ET for E3.5 embryos (ES cell)
- Pronuclear/IVF technology = E0.5 embryos
Why was this Challenge developed? Gap analysis

- Embryos cultured *in vitro* from E0.5 to E3.5 have extremely poor implantation rates
  ➢ Why: poor culture conditions?

- NSET using <E2.5 embryos have extremely poor implantation rates
  ➢ Why: not retained in the uterus?
Why was this Challenge developed? Business

• MLC performs ~2,500 ETs/year
  ➢ Worldwide ~0.25M embryo transfers/year

• Opportunities for efficiency gains
  ➢ Surgical ET is time consuming (30mins)
  ➢ Cost saving (£70 consumables/surgery)
Why was this Challenge developed? 3Rs drivers

- **Refinement in animal usage**
- **Reduction in adverse effects**
- **Huge worldwide impact if all surgeries were replaced with a suitable technique**
Current state of the art: Culture systems

- Tri-gas mini incubators
- Mini culture drops
- Specialised media preps:
  - KSOM (Millipore)
  - Origio Cleav/Blast
  - KSOMaa Evolve (Zenith)
  - Antioxidant & myo-inositol supplements
Current state of the art: Embryo transfer

- Surgical transfer: well understood
- Two alternatives
  - NSET (Paratechs: Steele, 2013)
  - TCET (ElimSprings Biotech: Cui, 2014)

$250 for box of 10

£120 for box of 10
Development of TCET at MLC

<table>
<thead>
<tr>
<th></th>
<th>3.5days in vivo (n=17)</th>
<th>3.5day in vivo + O/N culture (n=10)</th>
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<tbody>
<tr>
<td>Pregnancy rate (%)</td>
<td>88.24</td>
<td>80.00</td>
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<tr>
<td>Viable foetuses at E14 (%)</td>
<td>42.40</td>
<td>36.50</td>
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<tr>
<td>Implantation site (%)</td>
<td>56.80</td>
<td>64.86</td>
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Challenge deliverables

- Implantation rates of $\geq 40\%$: non-manipulated
- Implantation rates of $\geq 25\%$: post-manipulation
- Low cost
- Easily adapted to existing laboratory protocols
- Not technically challenging
Things we want to avoid

- Need for expensive/unique equipment
- Should not involve additional processes
- Must not compromise biosecurity
- Must not cause harm to the mice
Sponsorship in-kind provided by the MLC

- Expertise in mouse husbandry and genetics
- Surgical and non-surgical procedures
- In vitro/in vivo validation of test approaches
- Specific advice and guidance
- Introductions to other experts in the field
- Extensive network/outreach programme
Thank you for your attention

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