

## GENE DELIVERS ON THE 3R's

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We present three refinements within the Gene Delivery team; a service team at MRC Harwell specialising in the production of genetically modified mice. The aim of these refinements is to maintain our output but reduce the overall use of animals and embryos needed. We also wanted to ensure we were using best practice guidelines to avoid repeating procedures.

1) Quality vs quantity in superovulation - Good super-ovulations are often seen as those with highest yields, this has led to the use of young pre-pubescent females that can be consistently super-ovulated and mated to give high embryo yields. We believe that high embryo yields are not always a good outcome and that by over stimulating the reproductive system we are sacrificing the quality of the embryos produced. By using older females in the superovulation a better quality embryo is produced that is more robust and able to withstand all the techniques that we use to deliver CRISPR Cas9 reagents, resulting in improved birth rates.

2) Surgical clipping - Traditionally embryo transfer incisions have been closed using a surgical clip. This is removed 7 days post-surgery. The removal of the surgical clips can be problematic and stressful for the expectant female. With these issues in mind we wanted to make a refinement to this part of our embryo transfer procedure to reduce the loss of animals and valuable embryos and also to improve the healing process for the recipient female. We decided to trial a topical tissue adhesive and suture technique. With this we also created an incision healing guide to aid the technicians in dealing with any complications that may arise from this technique. This has resulted in a reduction in animals needing to be culled following surgery.

3) Non-Surgical Embryo Transfer (NSET) - The last of our refinements was to replace surgical embryo transfers for blastocyst injected embryos with a non-surgical approach. Surgical transfers require the pseudo pregnant female to be placed under anaesthesia and undergo an invasive procedure to implant blastocysts into the uterus. This would take up to 30 minutes to perform from start to finish. The NSET technique allows implantation of blastocysts by direct entry via the cervix. The procedure is quick, much less severe and the female recovers well.