



# GENE DELIVERS ON THE 3R'S

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## 1 Introduction

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 want to ensure we use best practice guidelines to avoid repeating procedures.

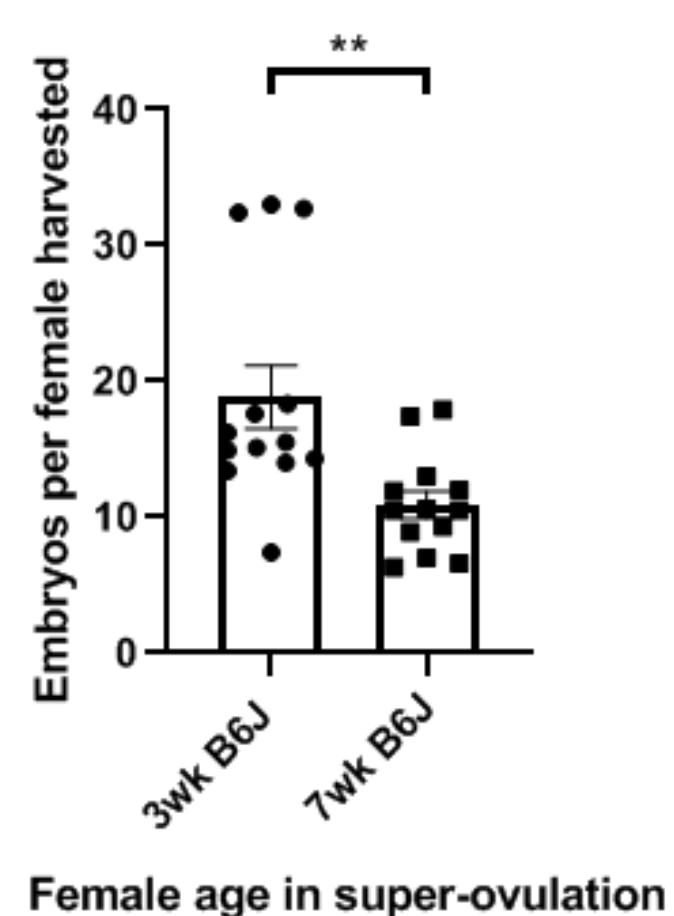
## 2 Quality vs Quantity in Superovulation

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.

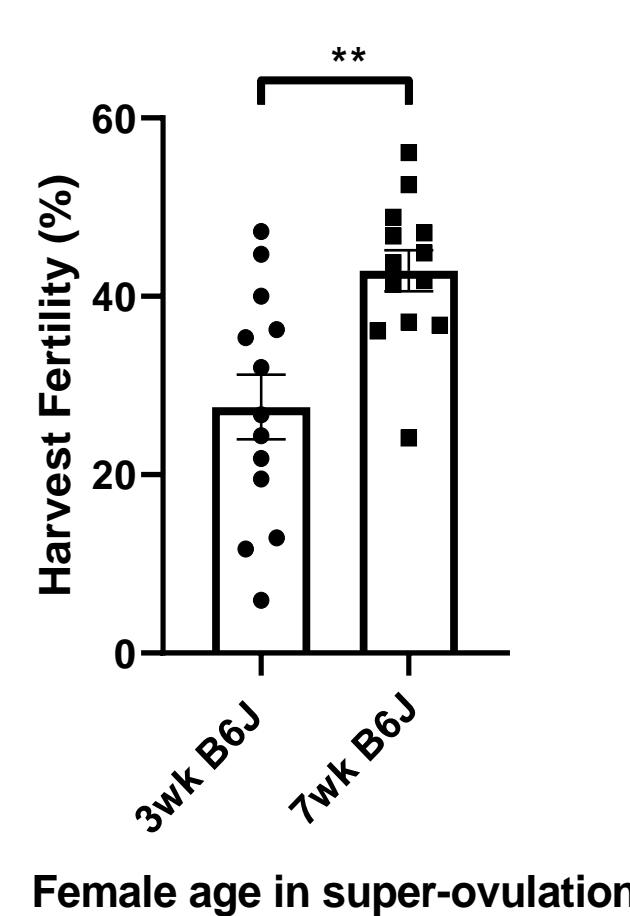
## 3 Quality vs Quantity Results



n = 13

Super-ovulation with 7wk old females gives significantly lower embryo numbers than with 3wk old females.

The harvest from 7wk old females has significantly higher fertility than that from 3wk old females. Taken together the data shows that the same number of mice at 3wk and 7wk will super-ovulate to give similar numbers of fertile embryos.



n = 13

(n=11 for the mutation rates as x2 projects had the offspring eaten in one control group)  
Data paired for Crispr-CAS9 project targeted.

Injected embryos from 7wk old females give significantly higher birth rates compared to 3wk old females.

There was no affect on the mutation rates.

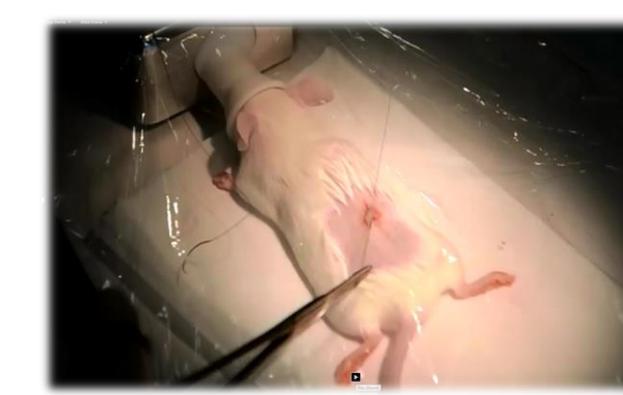
## 4 Surgical Clipping Replacement

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 GLUTure and suture technique. This involved using a scalpel to make the incision to create a single clean cut.



Post transfer of embryos, we place a single interrupted subcutaneous stitch to close the incision site and then cover this with a small amount of GLUTure.



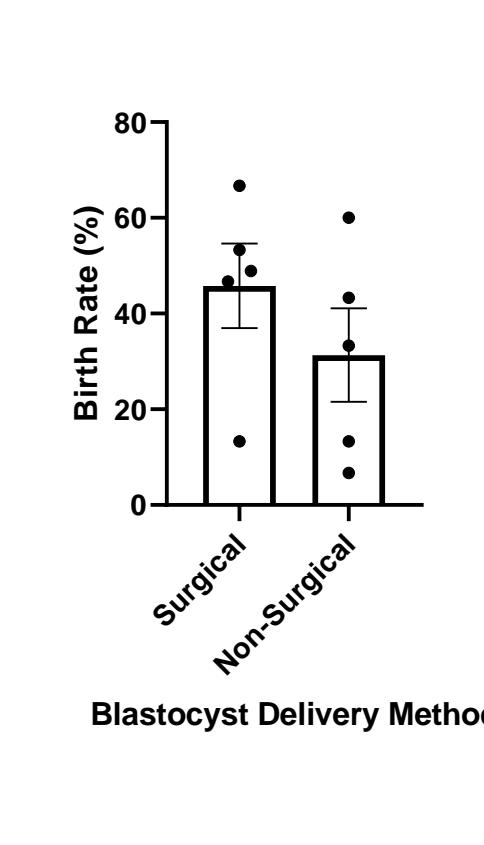
To accompany this technique we created an incision healing guide to aid the technicians in dealing with any complications that may arise from this technique. This has resulted in a reduction in animals needing to be culled following surgery.



Day 7- post surgery

## 5 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 around blastocysts into the uterus. This would take up to 30 minutes to perform from start to finish.



Blastocysts injected with ESC cells producing a common pool of injected blasts.

Pool of injected blastocysts split between surgical and non-surgical transfers.

No significant difference between the two transfer methods.



## Discussion

### Quality vs Quantity

Although the harvest may not yield as many embryos the rewards are far greater downstream with enhanced birth rates. Switching to using older females is also of benefit to the mice as the severity of the procedure is reduced, and young prepubescent females are no longer being expected to mate successfully.

### Surgical Clipping Replacement

The GLUTure suture technique is now the standard way we close all Embryo transfer incisions. This technique is quick to pick up and has a minimal time impact. With the added incision guide there are few complications with using this technique once competent.

### Non-Surgical Embryo Transfer (NSET)

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. We are also able to achieve the same results using this transfer technique.

## Acknowledgments

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