# OPTIMISING EMBRYOLOGY PROTOCOLS: COMBINING METHODOLOGIES TO REDUCE BOTH TURNAROUND TIMES AND NUMBER OF ANIMALS REQUIRED

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## 1 INTRODUCTION

In the mouse model, embryology (cryopreservation, rederivation, lineage expansion etc) and transgenesis services are mainly based on the use of pre-implantation embryos. The production and genetic modification of these embryos are still frequently carried out *in vivo* using sexual reproduction strategies (mating of animals with genotypes of interest). However, mating failures and the variability of fertilisation rates as a function of genetic background and environmental conditions (type of housing, season, food, noise, age of animals, etc.) lead to significant variability in results and prevent optimisation of animal resources. In addition, some processes require animals to be bred over several generations, which means that projects take a particularly long time to complete. The Charles River European Embryology Platform has been working continuously for several years to develop alternative *in vitro* procedures to sexual reproduction. Here we present some of the solutions that have been put in place and the benefits obtained in terms of reducing the number of animals used and project completion times.

## 2 IN VITRO FERTILISATION (IVF): A KEY TECHNOLOGY FOR EMBRYO PRODUCTION

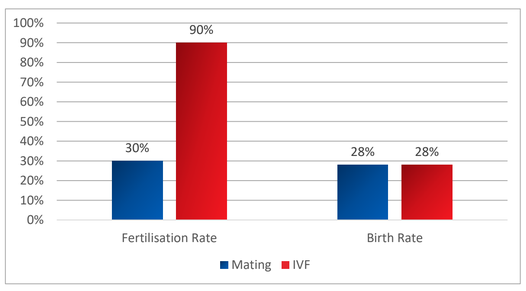



Figure 1: Comparison of IVF embryos vs natural mating with superovulation of females

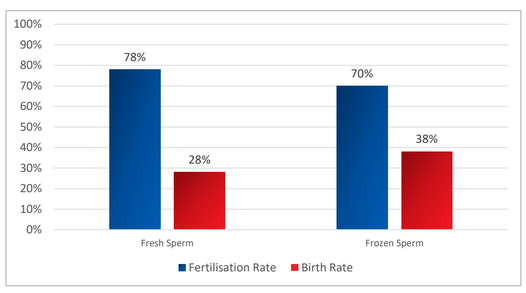



Figure 2. comparison of IVF performance with fresh vs. frozen sperm

### R as in Replacement

**IVF Benefits**

- Increased productivity: + 60% (Fig.1)
- Similar birth rate (Fig.1)
- Not affected by mating failure
- Robust performance

**Frozen sperm vs fresh sperm**

- Optimised birth rate: +17% (pups obtained per 100 embryos reimplanted)
- 1 single male is sufficient for most services
- Project security

*The data presented are based on a retrospective analysis from 2014 to 2021.*

### R as in Reduction

- Estimate by service : some examples on Table 1
- Estimate per year: > 4,000 mice including all services

| Service | Goal | # Mice by Services | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| Rederivation | 10 animals | 2 | 5 | 7 |
| Embryo Cryo | 300 embryos | 3 | 35 | 38 |
| Genetic engineering | Production of 100 embryos at the1-cell stage | 3 | 10 | 13 |

Table 1. Reduction in the number of animals per service

### R as in Refinement

**Laser-assisted IVF**

Increases the fertilisation rate compared to standard IVF

**Indications:** murine lines with altered sperm parameters (fresh sperm or cryostock)

**Benefits:**

- Avoids repetition of standard IVF
- Avoids wastage resulting from line loss
- Previously invested resources including animal resources
- Re-creation of the model

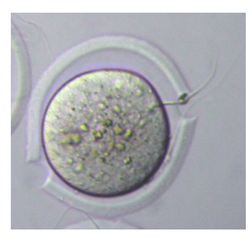
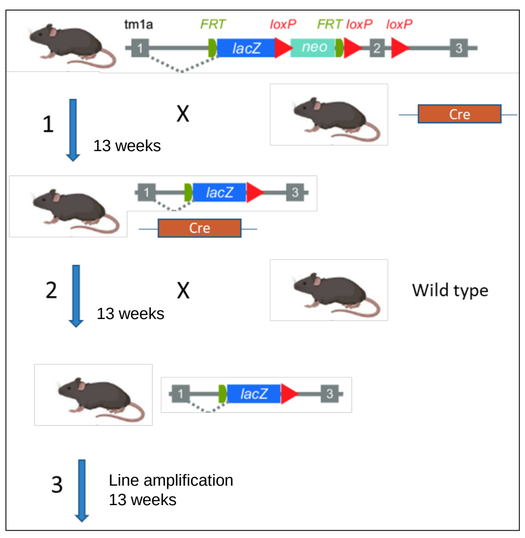


Figure 3. The laser precisely perforates the zona pellucida, making it easier for the spermatozoa to access the oocyte.

## 3 UBIQUITOUS MODIFICATION OF ALLELES BY RECOMBINASE

### Standard approach: mating

- Requires several generations of mating: very long lead time and use of a large number of animals
- Process highly affected by the fertility of the lines used
- Variable efficiency of allelic conversion depending on the type of recombinase, genetic background, promoter used, etc.
- Probable risk of modifying the genetic background of the line carrying the target sequences




### Optimised *in vitro* approach using Electroporation

**Reduced number of animals**

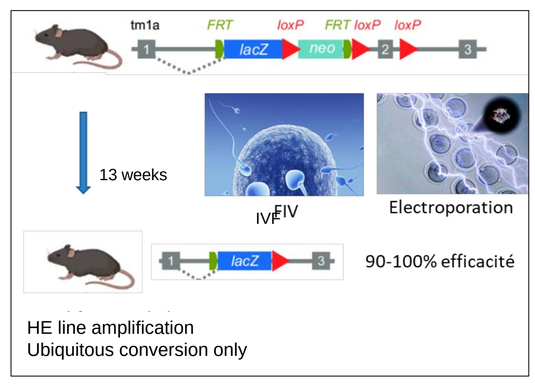
- 90-100% efficiency with Cre and Flp recombinases (Charles River data)
- No need to import and breed the line expressing the recombinase

**Reduced lead times**

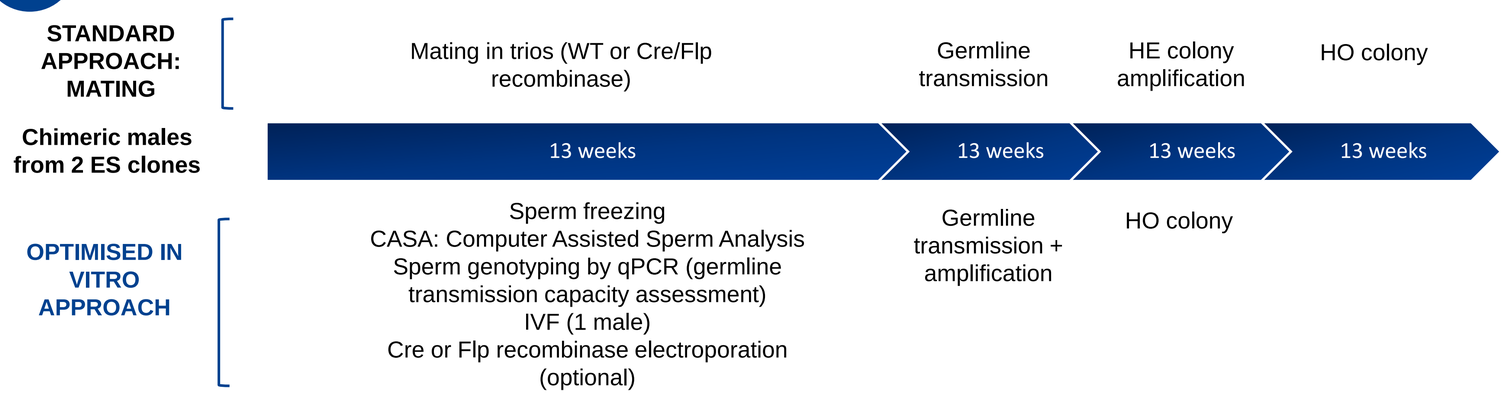
- 13 weeks vs 39 weeks to obtain the HE line

**Other positive points**

- The genetic background of the line remains unchanged
- No genotyping required to detect the transgene or allele expressing the recombinase
- Strategy easily applicable to other recombinases (e.g. Dre)




## 4 TARGETED TRANSGENESIS USING ES CELLS




**Reduction in the number of animals**

- Reduction in the number of chimeric males required
- Reduction in the number of F1 animals of no interest (genotype) euthanised

**Reduction in time required**

- Evaluation of the germline transmission capacity of chimeras from the 13th week of age
- Reduction in project duration by 25% up to the HE line

**Other positive points**

- Securing the project at an early stage by cryopreservation
- Reduction in the number of genotyping points
- *In vitro* recombination mediated by recombinases possible without increasing timelines

## 5 CONCLUSION

The development and optimisation of *in vitro* procedures for the production and genetic modification of pre-implantation mouse embryos has enabled our platform to significantly reduce the number of animals used, as well as project times. IVF has completely replaced animal mating for the production of embryos used in our embryology and transgenesis departments. In addition, sperm freezing allows us to obtain improved performance and greater model security. For model creation services, the use of IVF in combination with other *in vitro* approaches such as CASA, PCR genotyping of sperm and electroporation have also led to significant gains. It should be noted that the performance of the *in vitro* solutions used relies heavily on the operational excellence of the team. In this respect, regular review of the procedures and technologies, as well as an ongoing training programme, are essential. Finally, our results highlight the relevance of an ethical approach in the laboratory in terms of both animal welfare and quality of service.