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Is a reduction in embryo lysis possible using the MICRO-ePORE?

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1 - Introduction

CRISPR-Cas9 gene editing technology has increased our ability to produce Genetically Modified mice not only in a highly specific manner but also quickly and on demand. Establishments can easily design and produce the CRISPR guides and donors needed to engineer their mice. However despite these advances in the field there is still the need for the highly technical task of Pronuclear or Cytoplasmic microinjection to deliver the reagents, both of which can require a large number of embryos in order to reach the required number of founders.
One factor contributing to this high embryo usage is lysis due to damage to the embryo by the injection needle, this increases the level of embryo usage and the number of mice used to produce these embryos. We have been looking at the use of the MICRO-ePORE pinpoint cell penetrator, a devise designed to reduce the level of embryos lysis. The MICRO-ePORE enables the technician to inject under negative capacitance, which aids embryo survival by reducing any damage from the injection needle at the site of entry.

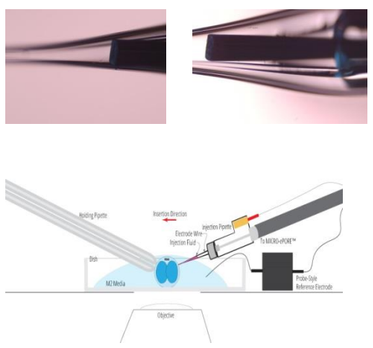
Considering The 3R's:
Replacement:
Use alternative to animals wherever possible.
Reduction:
Optimise the minimum number of animals wherever possible without compromising the data set.
Refinement:
Use best practice guidelines to ensure best use of all resources and to avoid procedures having to be repeated.

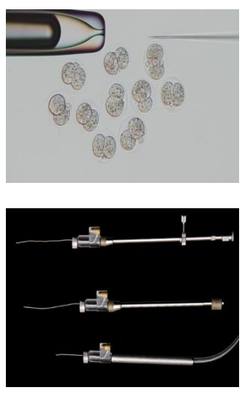
2 - Set up

We have used the MICRO-ePORE to aid cytoplasmic and pronuclear microinjection of one and two cell embryos.

A chloride silver wire is inserted into the injection needle ensuring it reaches the injection CRISPR mix, then on pressing the MICRO-ePORE foot switch just before injection the MICRO-ePORE then delivers a localized voltage signal to the site of injection which aids needle entry into the embryo with minimal damage. A reference electrode is also placed into the M2 media during microinjection which places the M2 at 0.0V to allow for the required voltage generated by the MICRO-ePORE.

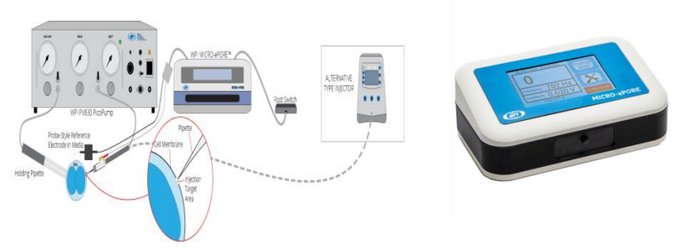
Audible and visual alarms are built into the MICRO-ePORE giving distinctive tones and colours on the MICRO-ePORE monitor when pressing the foot switch. If there is incorrect signal conduction then it gives a higher pitch tone and flashes red on the screen than if signal conduction is successful which gives a lower tone and flashes green on the MICRO-ePORE screen. This ensures that microinjection is carried out properly, the audible sound is useful as you cannot look at the screen at the same time of microinjecting.





3 - Technique

If you are familiar with a microinjection rig then the MICRO-ePORE is an easy setup however inserting the filament into the injection needle and mix can be tricky and time consuming.
In our hands the needles are pulled on a pipette puller and cut to an exact length (so that the MICRO-ePORE filament will reach the mix) then filled by capillary action and further backfilled using a microloader to ensure the filament makes contact with the mix. The settings used were the pre-set 4 program, 702HZ and 0.620V and we also use a foot pedal making the process much easier.

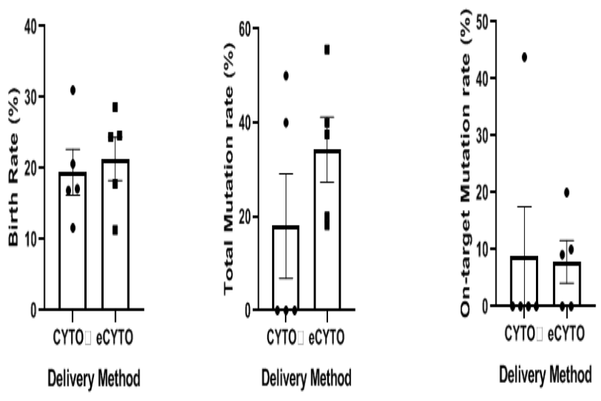


5 - Discussion

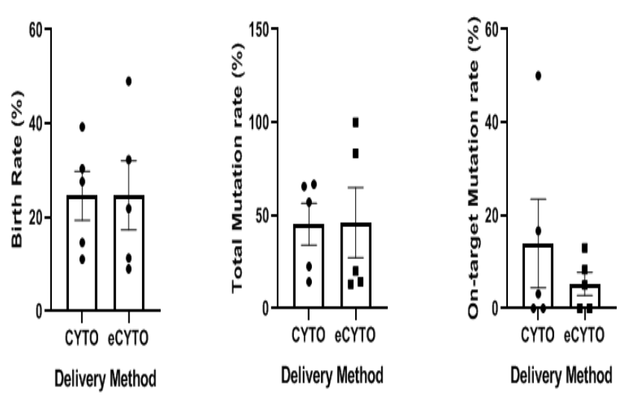
We used the MICRO-ePORE for Crispr-CAS9 targeted deletion, point mutations (oligo donors) and more complex projects (long single stranded donors). Our data shows intermittent lysis rate when using the MICRO-ePORE with no statistical difference in birth rates, mutation rates and on-target mutation rates when using the MICRO-ePORE for both pronuclear and cytoplasmic delivery.
Despite this it is a useful tool for handling difficult embryos or mixes that are difficult to inject and can lead to less Lysis rate.

4 - Results

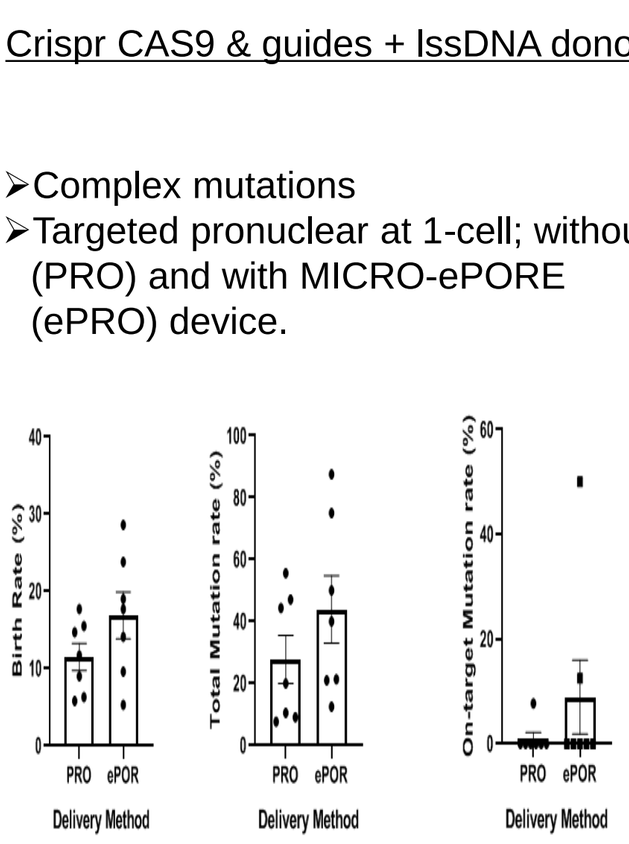
Crispr CAS9 & guides
- Deletion mutations
- Targeted cytoplasm at 1-cell; without (CYTO) and with MICRO-ePORE (eCYTO) device.



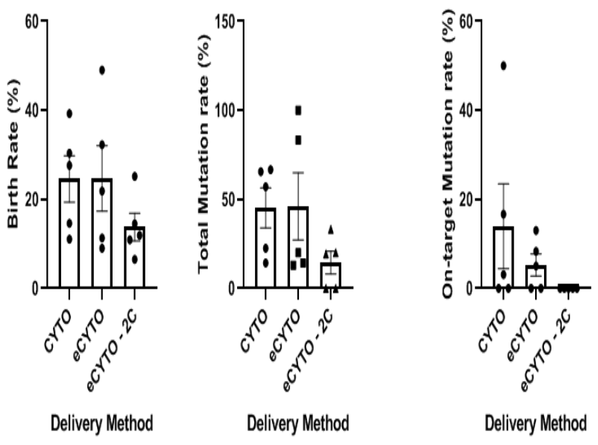
Crispr CAS9 & guides + oligo donor
- Point mutations
- Targeted cytoplasm at 1-cell; without (CYTO) and with MICRO-ePORE (eCYTO) device.



Crispr CAS9 & guides + IssDNA donor
- Complex mutations
- Targeted pronuclear at 1-cell; without (PRO) and with MICRO-ePORE (ePRO) device.



Crispr CAS9 & guides + oligo donor
- Point mutations
- Targeted cytoplasm at 1-cell or both cells of a 2-cell; without (CYTO) and with MICRO-ePORE (eCYTO) device.



Donor	Mix composition	Number of improved Lysis rate sessions using MICRO-ePORE	Number of not improved Lysis rate sessions using MICRO-ePORE
n/a	CYTO [50ng/ul CAS9 RNA, 6.25ng/ul guides]	2	3
oligo	CYTO [50ng/ul CAS9 RNA, 6.25ng/ul guides, 100ng/ul donor]	3	3
lssDNA	PRO [100ng/ul CAS9 RNA, 50ng/ul guides, 50ng/ul donor]	5	2

Acknowledgments

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