



# Kissing Goodbye to the Mouth Pipette

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## Introduction

Mouth pipetting has persisted as the standard technique for handling pre-implantation embryos in a large number of non-clinical laboratories. The convenience of the technique is undeniable but it remains the elephant in the room when it comes to health and safety inspections. As the only hand to mouth procedure tolerated in the modern laboratory, many institutions are looking around for viable alternatives.

Here we describe our experience of using Eppendorf CellTram manual micromanipulators for general embryo handling and EZ-Grip positive displacement pipettes for surgical embryo transfers in the mouse.

## Materials & Methods

Eppendorf CellTram manual micromanipulators were set up in accordance with the manufacturer's instructions and fitted with a hand drawn and polished glass capillary. Using this device up to 3 $\mu$ l can be loaded/dispensed quickly and in a controlled manner.



CellTram Micromanipulator

The EZ-Grip micropipettes were used as per the manufacturer's instructions and were fitted with a 170 $\mu$ m EZ-Tip. The EZ-Tip was reduced in length by 4cm to aid control during the embryo transfer procedure. For embryo transfer, 0.5 $\mu$ l media was introduced into the EZ-Tip, followed by an air bubble, the embryos, a second air bubble and more media to occupy the 1 $\mu$ l set volume. The bubbles confirmed the correct placement of the embryos during the procedure.



EZ-Grip

Surgical embryo transfers were conducted using a standard technique described by Behringer et al (2014) under gaseous anaesthesia. Analgesia was provided by injection of butorphanol and buprenorphine to give short and long lasting pain relief.

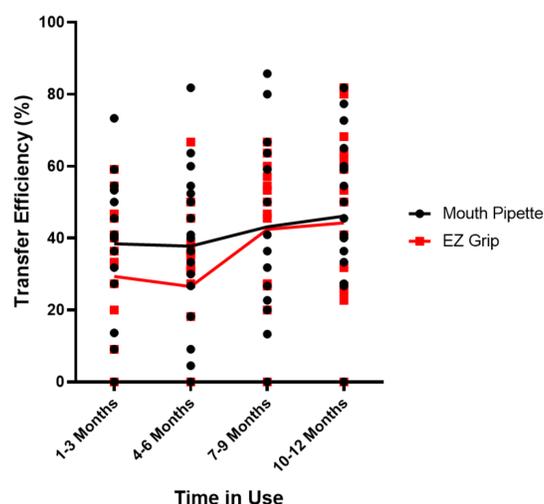
The embryo harvests and transfers were conducted as part of ongoing service work. No animals were used solely to generate data for this report.

All mouse work is overseen by the local animal welfare and ethical review body (AWERB). AAALAC accreditation was awarded to the Mary Lyon Centre in 2016.

## Results

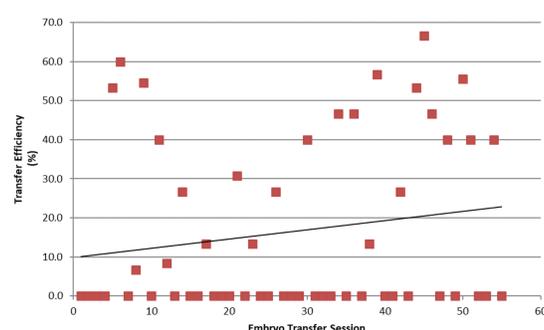
The preliminary data is very promising and on average we are recording an 83.9% pregnancy rate for embryo transfers using the EZ-Grip, compared to 93.3% for the mouth pipette. Plus, a 35.6% birth rate for EZ Grip compared to 41.4% for the mouth pipette. We anticipate making further progress as the technical staff become more familiar with the procedure.

Comparison Between Mouth Pipette and EZ-Grip on Embryo Transfer Efficiency



Embryo transfer efficiency from a technician experienced in using a mouth pipette. The length of the EZ tip was reduced by 4mm after 9 months of using the EZ Grip, aiding stability during the embryo transfer procedure. 1-3 months: EZ Grip (n = 17), MP (n = 21). 4-6 months: EZ Grip (n = 20), MP (n = 27). 7-9 months: EZ Grip (n = 16), MP (n = 18). 10-12 months: EZ Grip (n = 21), MP (n = 33).

Embryo Transfer Efficiency of Technician 'In Training'



Technician was in training and had never used a mouth pipette. Once pregnant, the females yielded a good number of pups.

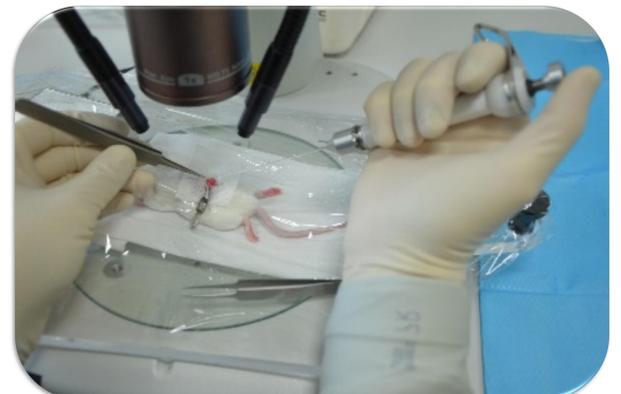
## Discussion

After some initial hesitance the use of the CellTram for general embryo handling has been broadly accepted and is the preferred method within the laboratory. After the initial outlay of procuring the equipment, the CellTram uses the same glass capillaries as the mouth pipette.



Micromanipulator in use for general embryo handling

Replacing mouth pipetting with the EZ Grip for embryo transfer has required more perseverance, but shortening the EZ tip has provided greater control and both improved embryo transfer success rates and user satisfaction.



EZ-Grip for use for embryo transfer

In conclusion, the two devices described offer the community viable alternatives to handling mouse embryos with a mouth pipette. They are relatively simple to use, inexpensive and completely eliminate the hygiene risks associated with mouth pipetting. However, they are not the only options available and interested parties are encouraged to explore alternative pipetting devices for themselves to find what works best for them.

Although we have not taken the bold step of discarding all remaining mouth pipettes, we hope not have to purchase any more in the future.

## Disclaimer

The authors have no vested interest in the commercialisation of either Eppendorf CellTram manual micromanipulators or EZ-Grip positive displacement pipettes.