A Technique for Facile and Precise Transfer of Mouse Embryos

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Abstract

Background: Successful Embryo Transfer (ET) technique is a fateful step of all efforts to achieve live births from in vitro produced embryos in assisted reproductive techniques or in knockout, transgenic or cloned animal projects. Small reproductive tract of mice and limitation of current techniques may not well satisfy the requirements for mass production of genetically modified mice. Genetic abnormalities of embryos, receptivity and uterine contractions, expulsion of embryos, blood, mucus or bacterial contamination on the transfer pipette tip, technical problems and even animal strain may affect embryo transfer outcome.

Methods: In this study, two techniques of embryo transfer in mice were compared. In conventional technique the oviduct wall was punctured with a 30-gauge needle and the loaded Pasteur pipette with embryos and medium was inserted into the hole. In new technique, embryos that were loaded in modified micropipette with minimal medium were transferred directly to the oviduct by manual piston micro-pump easily. Embryo viability was evaluated considering the percentage of live healthy newborns.

Results: Results of the two techniques were compared by t-test within the NPAR1WAY procedure of SAS software (ver. 9.2). The average live birth rates in the novel methods was significantly higher (42.4%) than the conventional method (21.7%, p<0.05).

Conclusion: In conclusion, using new embryo transfer technique improved birth rate by preventing embryos expulsion from the oviduct, saving time and easy transfer of embryos with minimum volume of medium.

Keywords: Embryo, Embryo transfer, Mice, Micropipette

Introduction

Fate of all efforts to give birth to an in vitro embryo produced from infertility treatment or especially from knockout, transgenic or cloning projects depends on successful Embryo Transfer (ET) technique 1. Small reproductive tract of mouse and positive pressure in the oviduct or uterus result in the discharge of freshly transferred embryo from their point of insertion. Moreover, limitation of current equipment and techniques and the necessity of sophisticated transfer skills mainly bring about unstable pregnancy rate of genetically modified transferred embryos.

Many factors such as genetic abnormalities of embryos, defects in uterine receptivity, uterine contractions, expulsion of embryos, blood or mucus on the catheter tip, bacterial contamination of the catheter, and retained embryos have all been associated with problematic and unsuccessful embryo transfers 2. Animal strain may affect embryo transfer outcome, for example, DBA/2J mouse strain that is widely used for biomedical research suffers from poor reproductive performance while F1 C57BL/6×DBA/2 (B6D2F1) and FVB/N strains are routinely used in ET programs. Heterosis, hybrid vigor, or outbreeding enhancement, is the improved or increased function of any biological quality in a hybrid offspring. Rose C. et al reported that after reciprocal embryo transfer in inbred DBA/2J, C3H/HeNCrl and outbred NMRI mice the highest embryo yield was achieved in outbred NMRI females, but embryo yields were similar in DBA/2J and C3H/HeN mice following...
superoovulation despite poor estrus cycle synchro-
nization in DBA/2J females.

The first oviduct transfer of mouse embryo was
reported by Tarkowski, and then Whittingham
demonstrated a widely-used method in rat and
mouse that turned into the routine procedures for
transfer of manipulated embryos. In convention-
al oviduct and uterus embryo transfer normally a
puncture hole or small incision is first made in the
wall of the uterus or oviduct by a 30-gauge nee-
dle for insertion of transfer pipeette. The tech-
nique described for uterine transfer was first de-
veloped by McLaren and Mitchie and extended
by Rafferty and Hogan. Various pregnancy rates
ranging from 0-75% after uterine transfer have
been reported. Also Michael A. et al reported a
transcervical embryo transfer method in mouse
using a Non-Surgical Embryo Transfer
(NSET™) device. The recent method is suitable
only for uterus embryo transfer while in some re-
search projects oviduct transfer is needed.

To arrest transferred embryos in the oviduct or
uterus, application of hyper-viscous transfer me-
dium, placement of the embryos into the endome-
trium or transfer of the embryos into the uterus
through the utero-tubal junction, which acts as a
natural barrier, have been utilized specifically.
Nonetheless, wide application of these modified
methods has been confined by the high-skill re-
quirements of the operating techniques. Zheng
Zhang et al described a modified embryo transfer
pipette with a syringe-like tip used with a mouth
pipette which is the same as conventional method.
They improved viability rate of mouse embryos
20% higher than conventional methods. In the
present study, we use a modified micropipette
with manual piston micro-pump that is more ef-
efctive, time-saving and easy to use with mini-
mum volume of transferred medium that elimi-
nates the need for professional technical training.

Materials and Methods

Preparation of modified transfer micropipette

A manual piston pump (Cell Tram oil®) (Figure 1)
and micropipettes (Kwik-fil®: Borosilicate
Glass Capillaries 1 mm outer diameter and 800μm
inner diameters) were used in this technique. For
washing, these intact micropipettes were put in
distilled water that was changed four times along
24hr. Then put in Milli-Q water for 24hr and were
dried in oven (180-200 °C) for 1-3 hr (Figure 2A).
A micropipette puller set (flaming brown, model-
P97, Sutter instrument Co., USA) pulled intact

micropipettes for inner diameter (I.D) of 150-
200 μm, and then by micropipette grinder EG-400
(Narishige, Tokyo, Japan) the tip was ground to a
flat and diagonal opening at a 45°C angle and a
sharp point (about 70-80 μm) was created at the
tip by microforge MF-900 (Narishige, Tokyo, Ja-
p) (Figure 2B).

Animals

We used eight-ten week old outbred NMRI
mice for all males (fertile and vasectomised) and
females (recipient and donor). They were main-
tained in a standard environmental and nutritional
condition.

Culture of collected embryos before ET

Donors (n=10) were superovulated using i.p.
injection of 5 IU HMG (Merional®: Human Men-
opausal Gonadotrophin, IBSA, Switzerland) and
after 45-47 hr, they were received 5 IU hCG
(Choriomon®: human Chorionic Gonadotropin,
IBSA, Switzerland) and mated with fertile male
(1:2, male: female ratio). Next day two-cell em-
byros were collected from donor with vaginal
plug through flushing of oviduct by M2 medium.
They were washed four times in M2 medium then
moved to KSOM medium drops under mineral oil
in 5% CO2 and 37°C incubator till ET.

Preparation of recipient mouse before ET

Recipients were mated with vasectomised
males (1:2 ratio) a day after donor mating without
superovulation protocol (HMG and hCG). Recipi-
ents with vaginal plugs were selected for embryo
transfer. Selected recipient mice (10 recipients/
group) were anesthetized with *i.p.* injection of ketamine (62.5 mg/kg body weight, Alfasan Co., Holland) and xylazine (6.25 mg/kg body weight, Alfasan Co., Holland). A 1 cm long skin incision was made parallel to the dorsal midline for exposing oviduct.

**Oviductal embryo transfer techniques**

**Conventional oviductal ET:** Conventional oviduct transfer was performed as control. A Pasteur pipette (outer diameter 5 mm, inner diameter approx. 4 mm, length 300 mm) was pulled on a flame smoothly to produce a tube with an internal diameter of 200-300 μm and a narrow shaft approximately 1.5-2 cm long. It loaded with a common arrangement of media (~ 10-15 μl) and three air bubbles to visualize successful transfer. The oviduct wall was punctured with a 30-gauge needle and the loaded pipette was inserted into the hole. The two-cell embryos (4 embryos/oviduct) were blown slowly into the oviduct until air bubbles became visible in the ampullae.

**Novel improved oviductal ET**

In this method first, two-cell stage embryos was loaded into modified embryo transfer pipette by manual piston pump (Cell Tram oil® or similar) under stereomicroscope with a common arrangement of media (≤ 1 μl) and air bubbles by a person. Then it was inserted directly into wall of the second coiled corner (counting from the ampullae) of the oviduct with the assistance of Watchmaker’s forceps under the other stereomicroscope by his colleague. First person turns the adjusting screw of pump to transfer embryo to oviduct. For evaluation of leakage and tracing the medium flow in oviduct, two control groups (10 mice/group) were selected and Trypan blue solved in M2 medium as a visible dye inserted into mice oviducts using conventional (10-15 μl) or novel (1 μl) method (Figure 3A). Oviduct was returned into the abdomen after dye injection and after some minutes it was exposed again to ensure if dye leakage occurred (Figure 3B). Embryo viability was evaluated by calculating the percentage of live healthy pup as numbers of live healthy pup divided by number of transferred embryos, in each mouse. The results of these two treatments were compared by t-test within the NPAR1WAY procedure of SAS software (ver. 9.2).

**Results**

In this improved method sharp tip of modified embryo transfer pipette can be inserted easily through the wall of the oviduct, so there is no need to puncture the oviductal wall by needle before insertion of pipette. Recently, Chin and Wang performed uterine transfer from the oviduct side of the utero-tubal junction and achieved a 100% implantation rate because of their success in preventing the embryos from flowing out of the incision when they withdrew the transfer pipette.13

Using the mentioned pump equipped with a micro-adjusting screw launches a gentle, fine, fast and exact transferring instruments for oviductal ET with minimal transferred medium to the oviduct compared with conventional method (≤ 1 μl vs. 10-15 μl, respectively). Tracing inserted dye into the oviduct showed that all of the transferred embryos would remain in the oviduct lumen (Figure 3). The pregnancy rate of unmanipulated zygotes after oviduct transfer ranges from 45-80% with an average number of transferred zygotes of 25-30.14 In this study the average live birth rates in novel methods was significantly higher (42.4%) than conventional method (21.7%; Table 1; Figure 4; p<0.05). The high viability rate of transferred embryo in novel technique might be attributed to time-saving process with fewer traumas and bleeding in oviduct, effective blockage of outflow of the medium from insertion site, and less transferred medium with embryos. These results were consistent with the results of Englert *et al* that reported a 33.3% pregnancy rate with excellent...
transfer technique, whereas "bad" transfers yielded a 10.5% pregnancy rate in human. Similarly, Mansour et al showed that difficult embryo transfers had a significantly lower pregnancy rate and implantation rate (4 and 1%, respectively) compared with easy transfers (20.4 and 6.7%, respectively). Others have reported a strong correlation between the ease of embryo transfer and pregnancy. Successful embryo transfer requires manual dexterity for introducing embryos into the reproductive tracts and preventing discharge of the transferred embryos from it; however, the latter consideration is often ignored.

Conclusion

In conclusion, utilization of this more user friendly and less invasive tool can greatly improve the success of embryo transfer and other techniques such as spermatogonial stem cell transplantation in rete testis and so on that need fine and exact transfer instruments.

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References