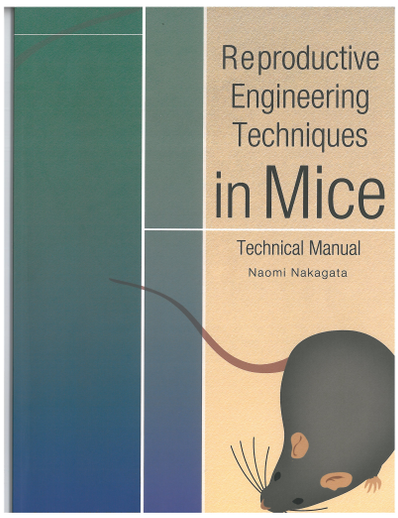
Reproductive Engineering Techniques **in Mice**



Technical Manual
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Preface

In recent years, the number of genetically engineered mice being produced has increased dramatically. Moreover, the rapid progress in the development of genome-editing (TALEN and CRISPR/Cas9) techniques for molecular biology research has been remarkable, so much so that a genetically engineered mouse strain can be produced easily in a few months. Production has been supported by reproductive engineering techniques such as *in vitro* fertilization, embryo and sperm cryopreservation, and embryo transfer techniques. Such techniques have become invaluable peripheral technologies, and their use is expanding rapidly.

This rapid expansion has led to the publication of many technical manuals relating to reproductive engineering techniques in mice hereto. However, mouse reproductive engineering techniques mainly involve delicate operations under a stereoscopic microscope, which means that a sufficiently detailed technical manual has not yet been published.

With that in mind, in this book we aim to create a mouse reproductive engineering technique manual that can be easily understood by anyone. We included a generous number of diagrams, photographs and videos in our manual, and explained each step of each reproductive engineering technique as clearly and thoroughly as we could. We sincerely hope that our manual will become the definitive guide for students, technicians, researchers and other people wishing to study mouse reproductive engineering techniques.

Naomi Nakagata

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* Please see page 90 for details.

7-1 Vasectomy for the Creation of Sterile Males

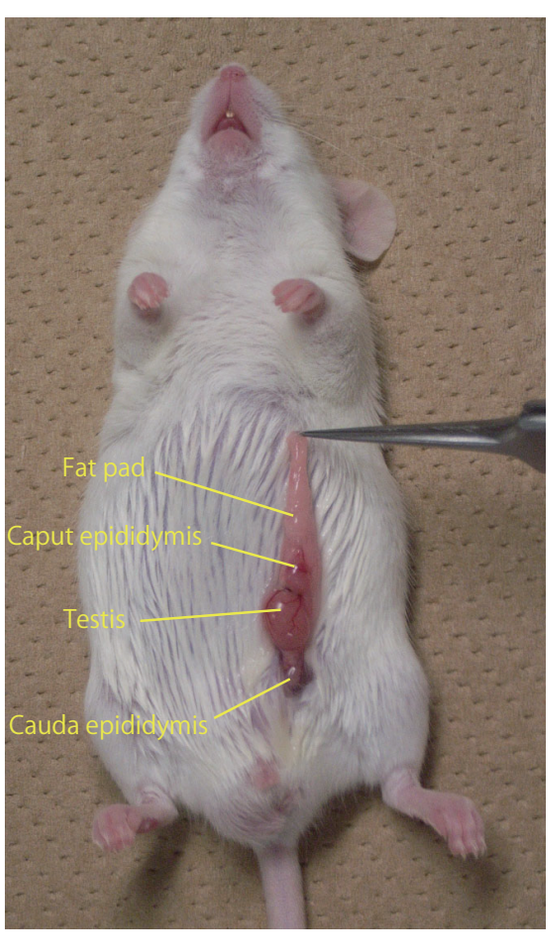
Materials and Equipment

1. Male mice (5 weeks old)
2. Anesthetic
3. Fine scissors
4. Pair of watchmaker's #5 forceps
5. Wound clip (Autoclip 9 mm; Clay Adams Cat. No. 427631) and clip applicator (Mik-Ron Autoclip Applier; Clay Adams Cat. No. 427630)
6. Hot plate (37℃)

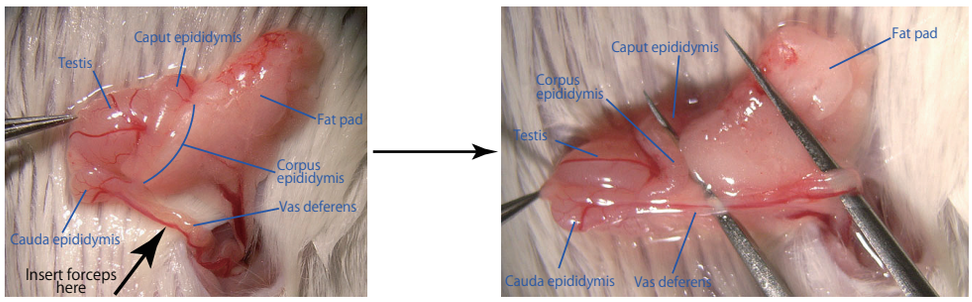
Procedures

Vasectomy

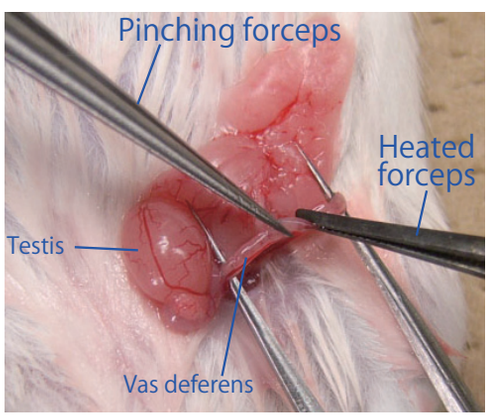
1. Anesthetize a male mouse.
2. In accordance with the conventional procedure, make a median incision. The incision should start at a point level with the top of the hind leg, and extend approximately 1 cm from this point towards the head of the mouse. After making the incision, pull out the testis, the epididymis and part of the vas deferens from the abdominal cavity.

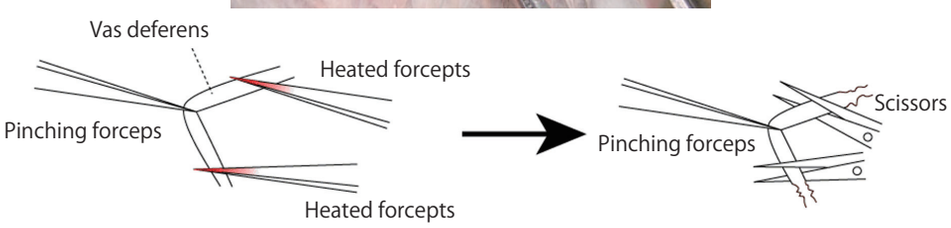


3. Pick up the vas deferens with one pair of forceps, and insert a second pair of forceps under the vas deferens to separate it from the connecting tissue.



4. Hold the vas deferens with a pair of pinching forceps, then cauterize the vas deferens at two locations using a second pair of heated forceps, as shown in the diagram below.
5. Cut the part of the vas deferens that lies between the two cauterized regions.





6. Push the testis, epididymis and part of the vas deferens back into the abdominal cavity, then close the wound using wound clips.
7. Repeat steps 2-6 for the other testis.

References

1. Nagy A., Gertsenstein M., Vintersten K., and Behringer R. 2003. Manipulating the Mouse Embryo, A Laboratory Manual (Third edition). *Cold Spring Harbor Laboratory Press.* ISBN 0-87969-591-9.

Comment

After completing the procedure, house each mouse individually. If the mice are housed in a group, they are likely to fight and some of them may be killed. The vasectomized males will be ready for use from as early as 8 weeks of age.

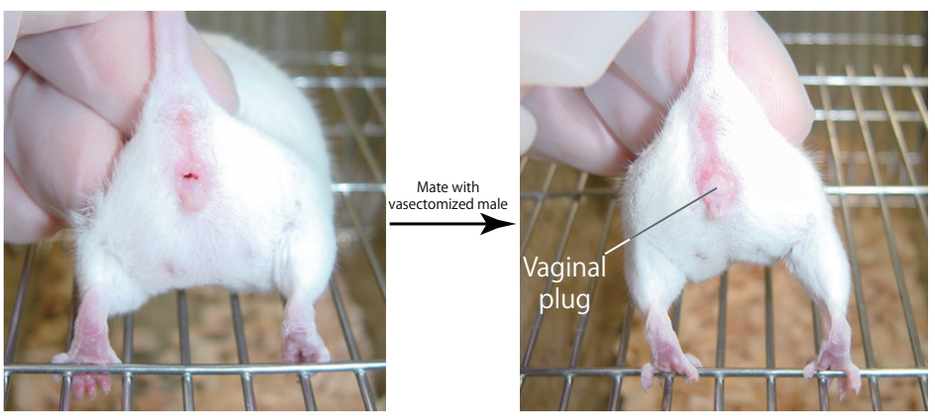
7-2 Embryo Transfer into the Oviduct

In our laboratory, we transfer 2-cell embryos through the wall of the oviduct of pseudopregnant recipients. This procedure is much easier and simpler to conduct than the conventional embryo transfer procedure, and is therefore suitable for inexperienced users.

Materials and Equipment

1. Female mice on Day 1 of pseudopregnancy (the day on which a vaginal plug is observed).

[Appearance of the vagina in proestrus] **[Vaginal plug]**

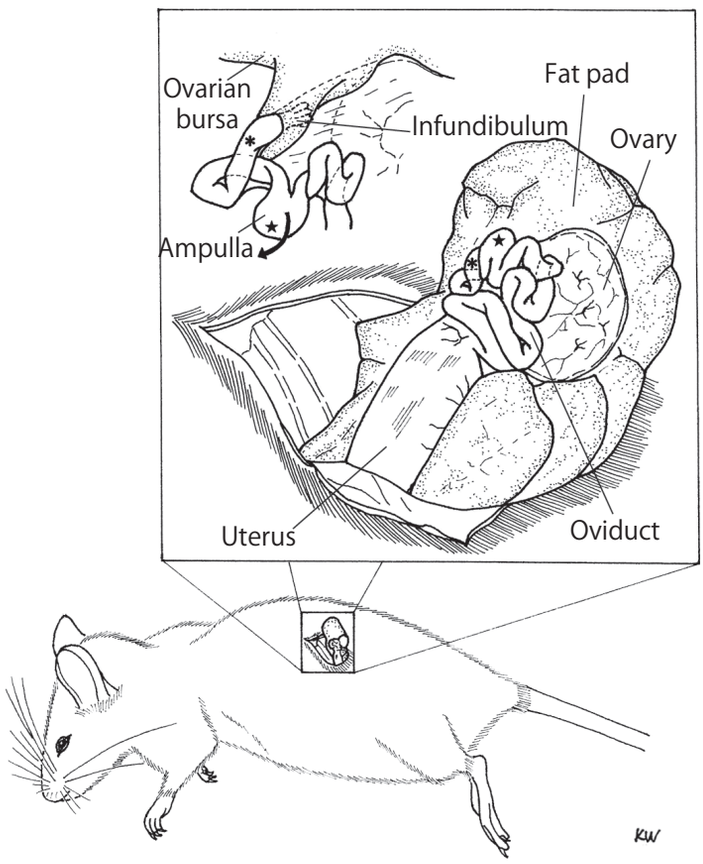


2. Anesthetic
3. Micro-spring scissors (5 mm blade)
4. Pair of watchmaker's #5 forceps
5. Serrefine clamp
6. Wound clip (Autoclip 9 mm; Clay Adams Cat. No. 427631) and clip applicator (Mik-Ron Autoclip Applier; Clay Adams Cat. No. 427630)
7. Plastic dish (35 mm X 10 mm Cat. No. 430588; CORNING)
8. Glass capillaries for embryo transfer and handling
9. Hot plate (37℃)

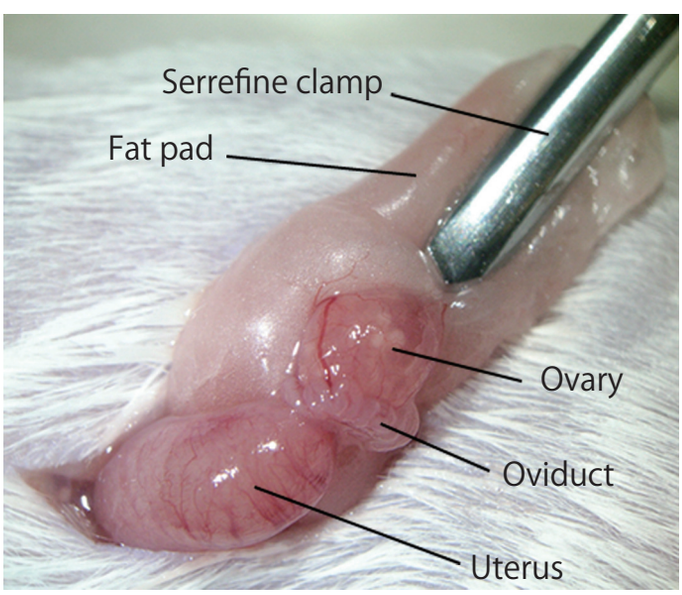
Procedures

Preparation of Mice

1. Anesthetize a female mouse.
2. Pull out the ovary, oviduct, and part of the uterine horn.



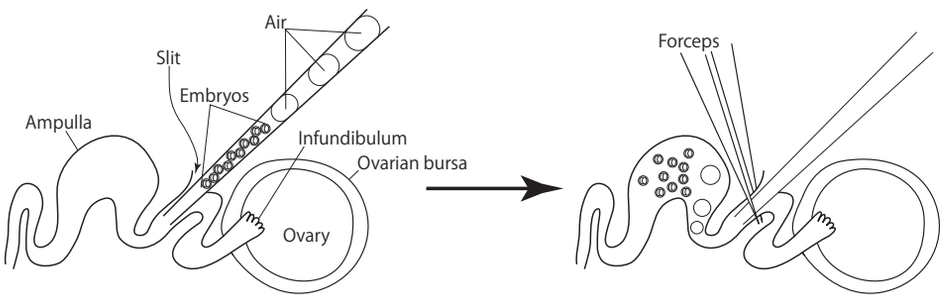
3. Clip a serrefine clamp onto the fat pad which is attached to the ovarian bursa.



Positioning of Oviduct

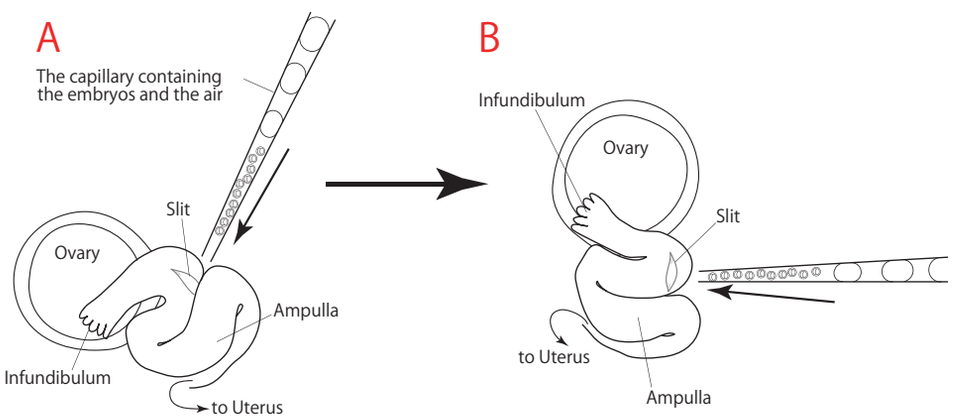
As indicated in the diagram below, embryo transfer into the oviduct is carried out by cutting the oviduct, inserting a capillary thereinto and expelling embryos towards the ampulla.

[Schematic Diagram]



Unfortunately, the oviducts of mice are small and the ducts are folded in a complicated manner, as shown in the schematic diagram of an exteriorized oviduct below (A). This makes it very difficult to insert the capillary into the oviduct towards the ampulla, because the insertion is made from above.

To make this procedure easier, position the oviduct by changing the position of the serrefine clamp and the mouse before starting the operation (B).



1. Observe the oviduct under a stereomicroscope and confirm the position of the infundibulum and ampulla using the tip of a set of forceps, or by changing the position of the serrefine clamp.
2. Position the oviduct by changing the position of the serrefine clamp and the mouse.

Note

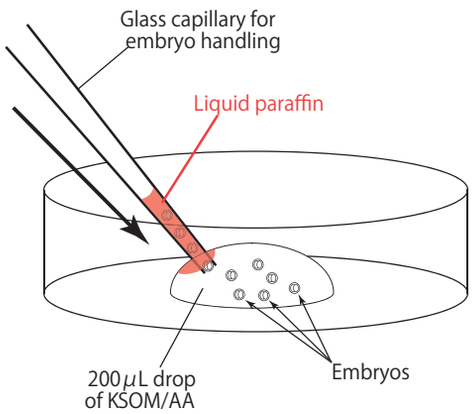
Because the folds in the oviduct vary between each mouse, look closely and adjust the position of the oviduct to make easier to work on.

Comment

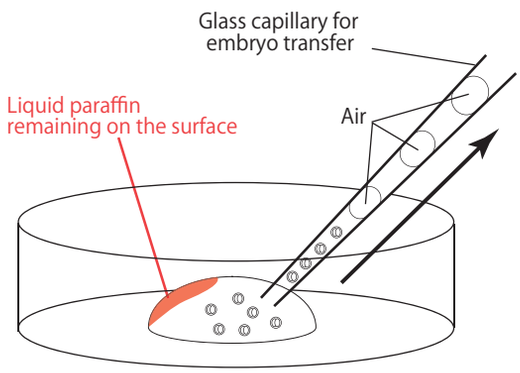
If you are left-handed, position the oviduct so that you can easily carry out the procedure with your left hand.

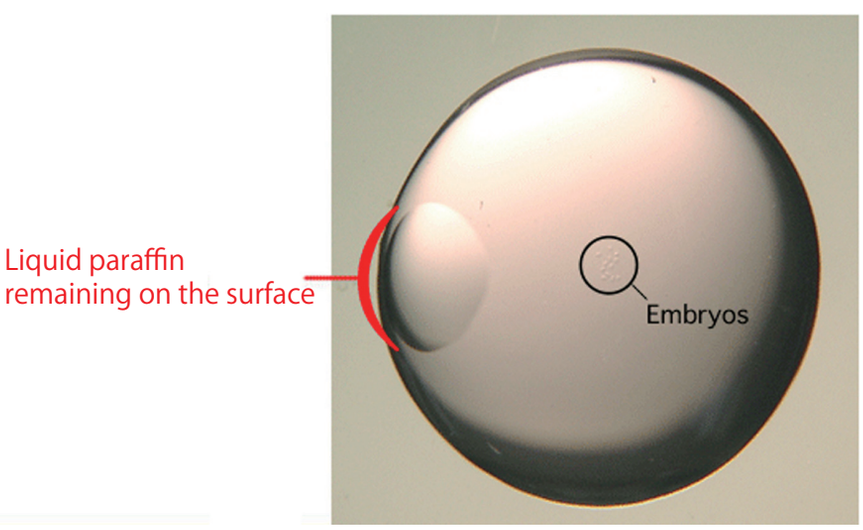
Preparation of Embryos and Glass Capillary

1. Make a 200 µL drop of KSOM/AA in a dish (without liquid paraffin), and introduce 20 embryos into the drop.



2. Aspirate air and medium in alternate intervals of 2-3 mm into a glass capillary in preparation for embryo transfer. Draw ten embryos into the glass capillary.





Comment

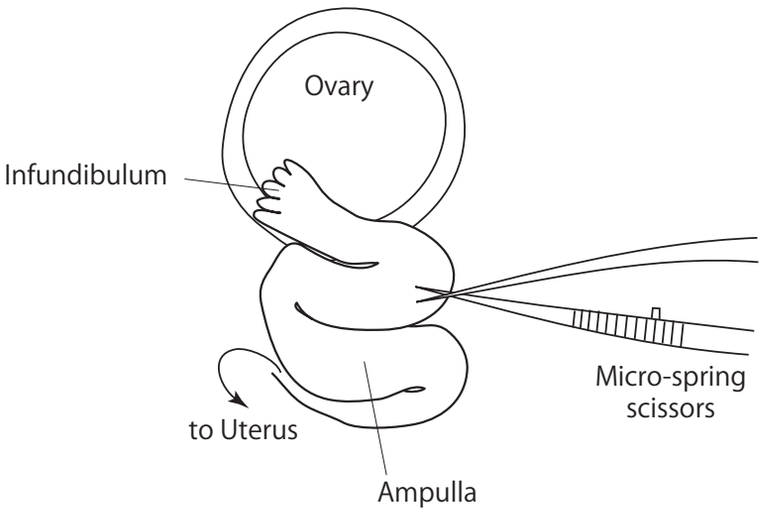
When the glass capillary is first inserted into the drop, some liquid paraffin will remain on the outer surface of the drop as shown below.

The embryos should be drawn into the glass capillary from the opposite side of the drop to avoid sucking up any liquid paraffin.

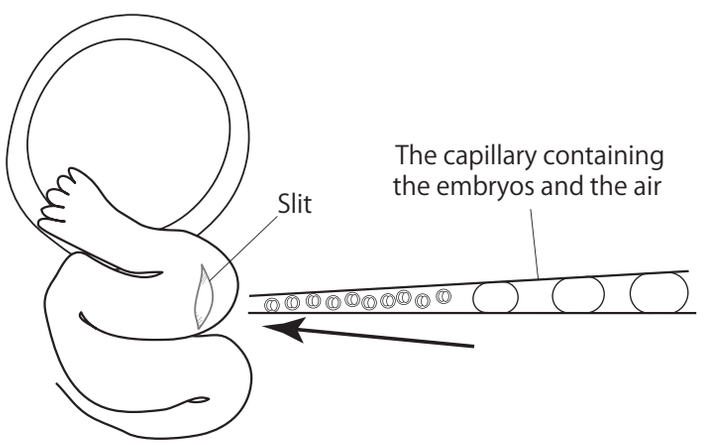
Evidence suggests that liquid paraffin which passes into the oviduct may have adverse effects on the development of the embryos into offspring.

Embryo Transfer

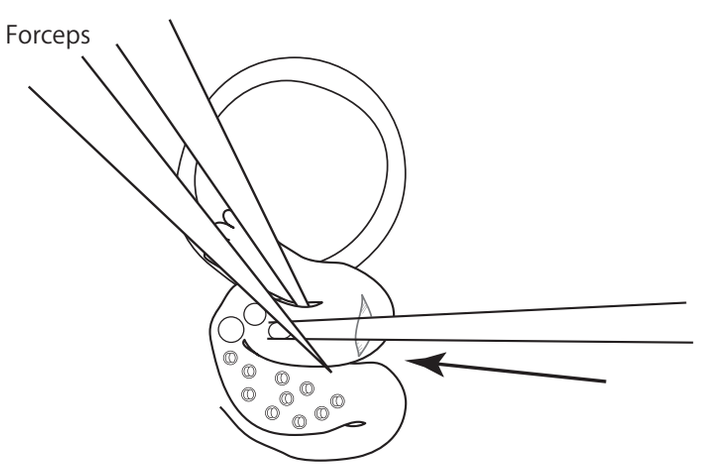
1. Using a pair of watchmaker's #5 forceps and micro-spring scissors, dissect the wall of the oviduct between the infundibulum and ampulla.



2. Insert the tip of the capillary containing the embryos into the slit, then push the capillary further into the slit towards the ampulla.



3. Use the forceps to hold the portion of the oviduct into which the capillary was inserted.
4. Expel the embryos and 2-3 of the air bubbles into the ampulla.



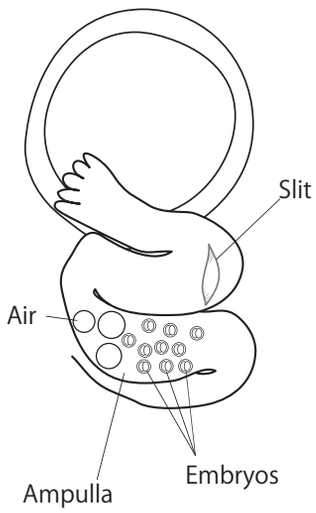
Comment

If performed successfully, you should be able to see air bubbles through the wall of the ampulla.

Note

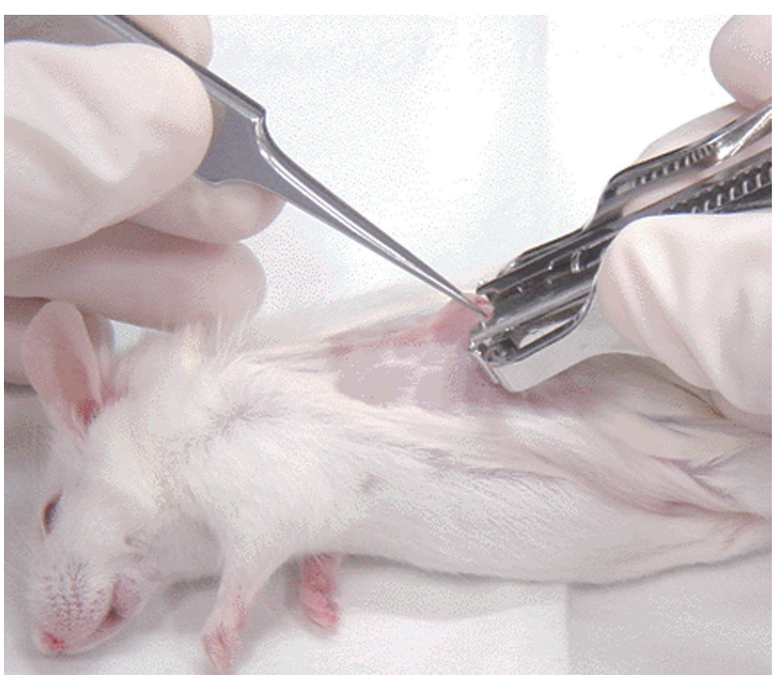
If you cannot expel the embryos and the air bubbles into the oviduct, pull the capillary back out just a little from the slit and expel them again.

5. Withdraw the capillary gently from the slit.



[Transferring Embryos into the Oviduct] No. 17-01

6. Push the ovary, oviduct and uterine horn back into the abdomen and close the wound using wound clips.



7. Repeat the process to transfer the remaining 10 embryos into the other oviduct as described before.
8. Keep the mice warm on a 37℃ warming plate until they recover from the effects of anesthesia.

References

1. Nakagata N. 1992. Embryo transfer through the wall of the fallopian tube in mice. *Exp. Anim.* **41**: 387-388.
2. Nagy A., Gertsenstein M., Vintersten K., and Behringer R. 2003. Manipulating the Mouse Embryo, A Laboratory Manual (Third edition). *Cold Spring Harbor Laboratory Press*. ISBN 0-87969-591-9.

Note

Transfer the embryos after adjusting the position and direction of the oviduct. If the oviduct is aligned parallel to the capillary, then it will be easier to insert the capillary into the oviduct.

7-3 Embryo Transfer into the Uterus

Materials and Equipment

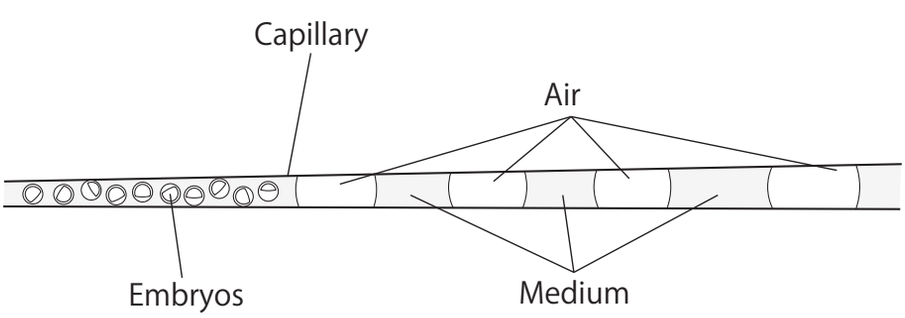
1. Female mice on Day 3 of pseudopregnancy (Day 1 is the day on which a vaginal plug is observed)
2. Anesthetic
3. Fine scissors
4. Pair of watchmaker's #5 forceps
5. Serrefine clamp
6. 27 gauge needle
7. Wound clip (Autoclip 9mm; Clay Adams Cat. No. 427631) and clip applicator (Mik-Ron Autoclip Applier; Clay Adams Cat. No. 427630)
8. Plastic dish (35 mm X 10 mm Cat. No. 430588; CORNING)
9. Glass capillary for embryo transfer
10. Hot plate (37℃)

Procedures

Embryo Transfer

Prepare recipient mice, embryos (8-cell to blastocyst stage) and a glass capillary as per the method used for embryo transfer into the oviduct.
(Please refer to the chapter of Embryo Transfer into the Oviduct on page 67.)

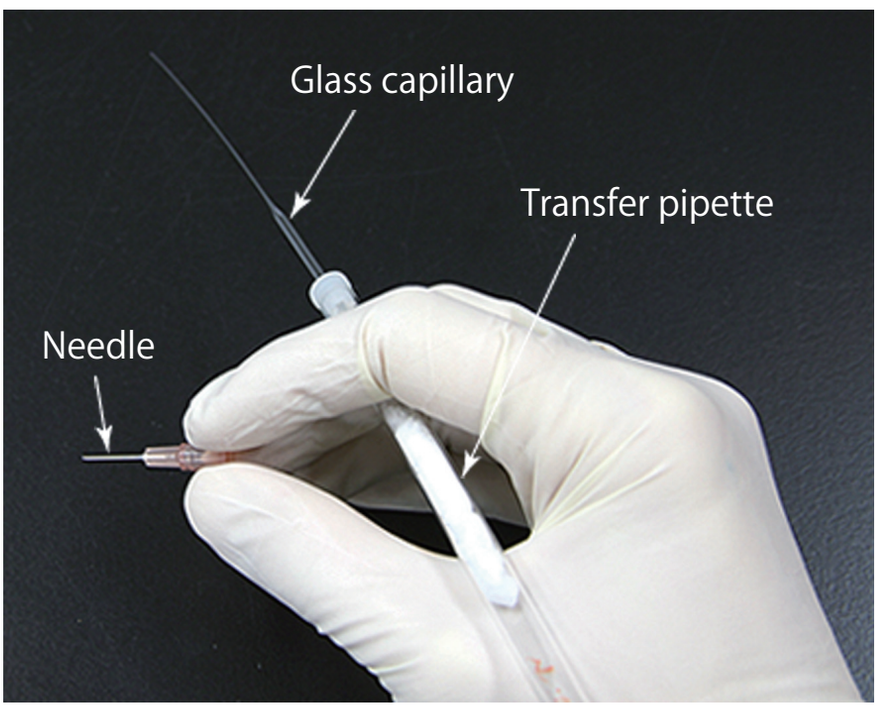
1. Pull out the ovary, oviduct and part of the uterine horn as per the conventional procedure.
2. Clip a serrefine clamp onto the fat pad which is attached to the ovarian bursa.
3. Aspirate air and medium in alternate intervals of 2-3 mm into a glass capillary in preparation for embryo transfer. Draw ten embryos into the glass capillary as indicated in the diagram below.



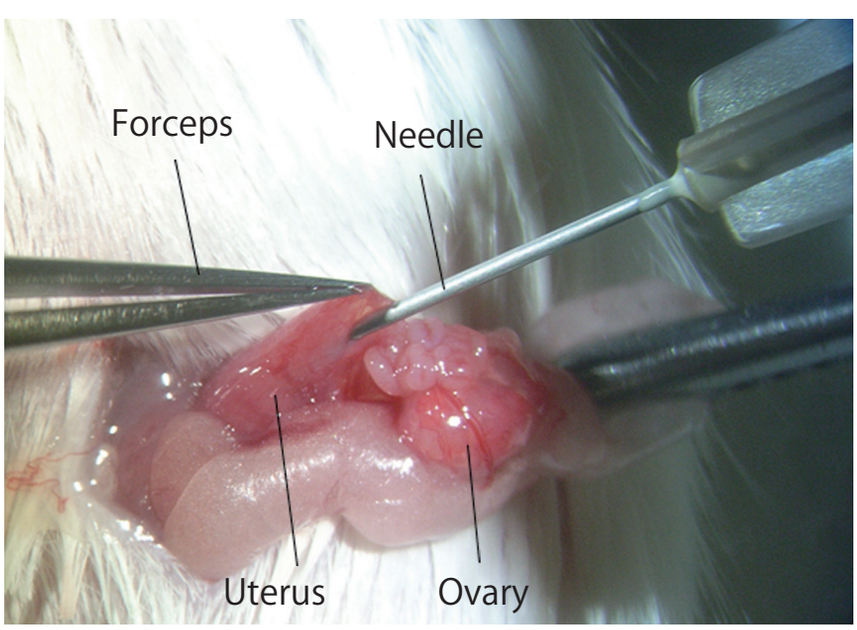
Note

When preparing the glass capillary, avoid placing the glass capillary into any liquid paraffin.

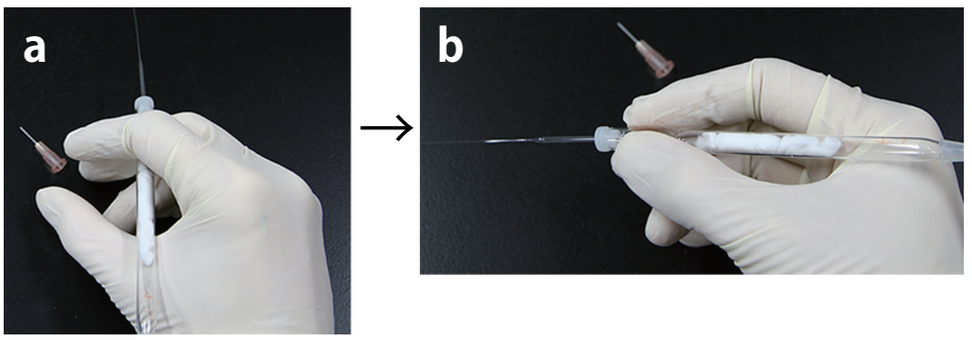
4. Hold a 27 gauge needle and the transfer pipette as shown in the picture below, and simultaneously look at both the tip of the needle and the uterus under a stereomicroscope. Be sure to look at the needle and the uterus simultaneously under the same stereomicroscope in order to confirm the position of the needle in relation to the uterus.

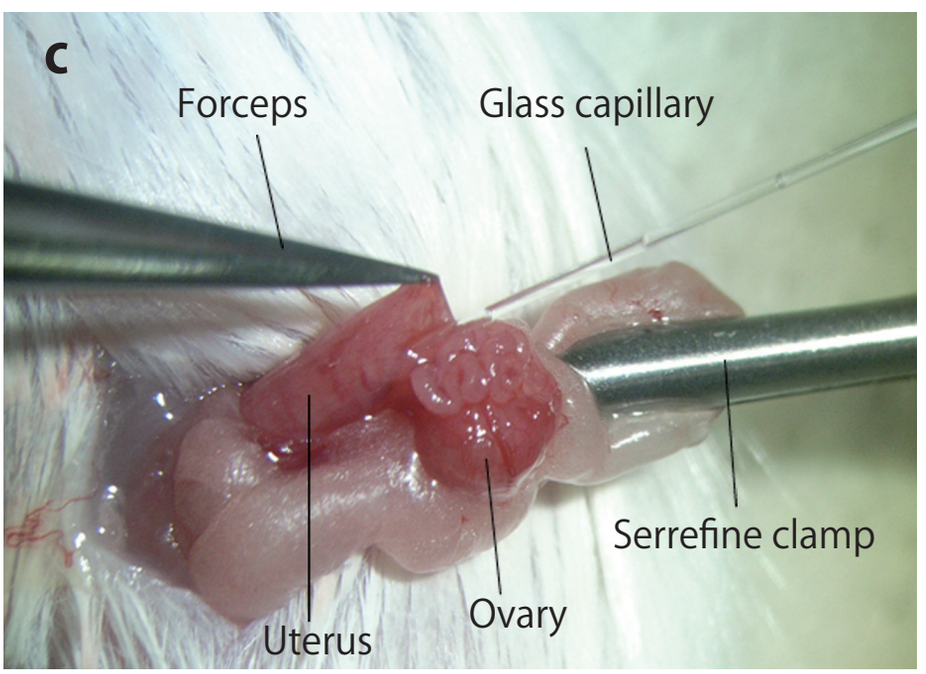


5. Gently hold the top of the uterine horn using fine forceps, and insert a 27 gauge needle into the wall of the uterus as far as the uterine cavity.



6. Release the needle and hold the transfer pipette as shown in diagrams a and b. Insert the tip of the capillary containing the embryos and air bubbles deep into the uterine cavity via the hole that you made with the needle, as shown in diagram c.





7. Expel the embryos into the uterine cavity along with 2-3 air bubbles.
8. Gently withdraw the capillary from the hole.

[Embryo Transfer into the Uterus] No. 18-01

[Performing the Operation] No. 18-02

Note

You should hold onto the top of the uterine horn and keep watching the hole made by the needle until you complete embryo transfer. If you avert your eyes from the hole before completing this procedure, it may be difficult to find the hole again.

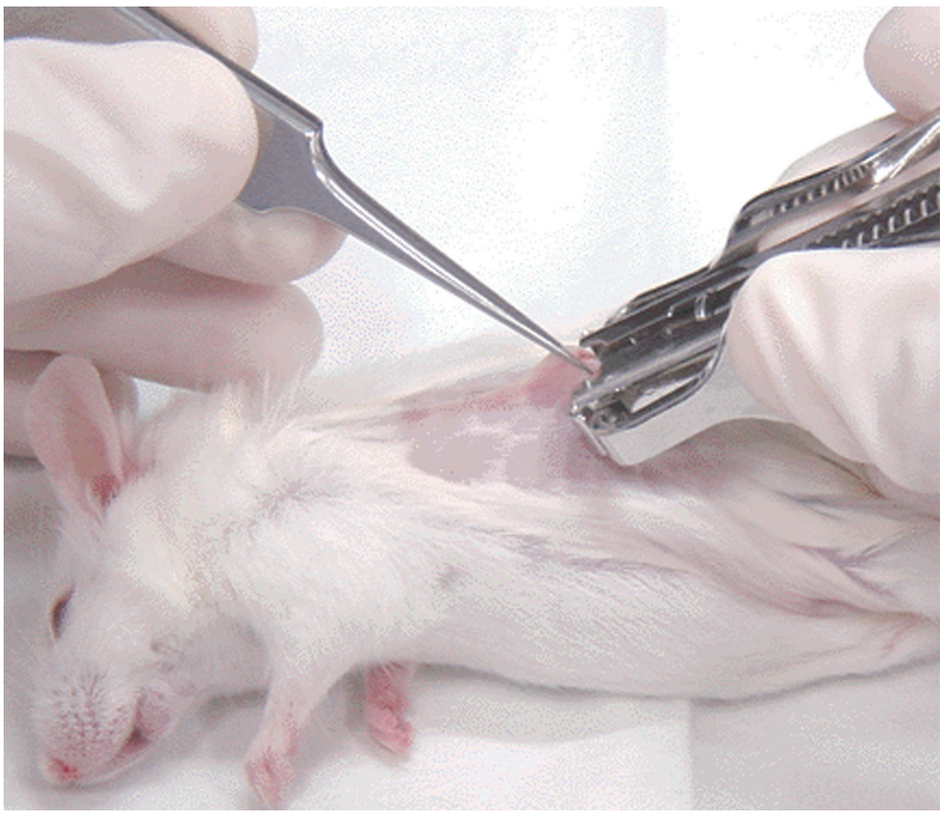
Note

If you cannot expel the embryos and the air bubbles into the uterine cavity, pull the capillary back out just a little from the hole and expel them again.

Note

To help you keep an eye on the hole made with the needle, you should hold both the needle and the transfer pipette in your dominant hand before you start the procedure.

9. Push the ovary, oviduct and uterine horn back into the abdomen and close the wound using wound clips.



10. Repeat the process to transfer 10 embryos into the other uterine cavity as described before.
11. Keep the mice warm on a 37°C warming plate until they recover from the effects of the anesthesia.

References

1. Nagy A., Gertsenstein M., Vintersten K., and Behringer R. 2003. Manipulating the Mouse Embryo, A Laboratory Manual (Third edition). *Cold Spring Harbor Laboratory Press.* ISBN 0-87969-591-9.

7-4 Caesarean Section and Fostering

A Caesarean section should be performed if a pregnant recipient has not given birth to babies by the estimated date of delivery.

Materials and Equipment

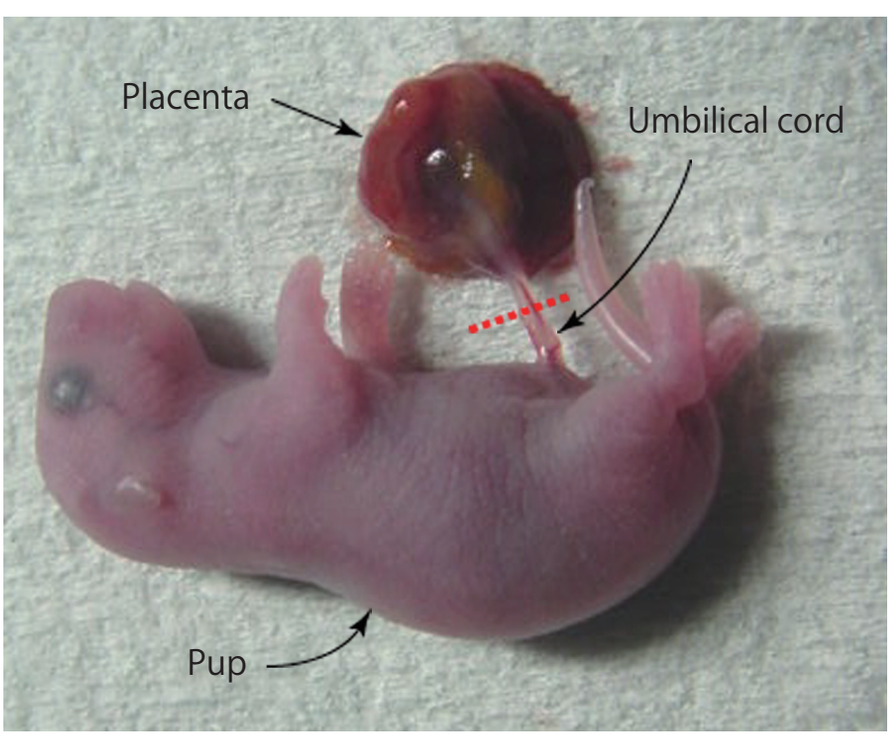
1. Foster mother (a foster mother is a female that has given birth on either the same day as, or the day preceding, the estimated date of delivery of the pregnant female)
2. Fine scissors
3. Pair of watchmaker's #5 forceps
4. Hot plate (37℃)
5. Pregnant female mouse

Procedures

Caesarean Section

1. Sacrifice the pregnant female and wipe the abdomen with a piece of cotton that has been soaked thoroughly in 70% ethanol.
2. Immediately open the abdomen and remove the uteruses containing the pups with a pair of fine scissors.
3. Place the uteruses on a paper towel, and cut through the uterine wall.
4. Quickly remove the pups from the yolk sac and amnion and cut the umbilical cord.

[Cutting the Umbilical Cord]



5. Use fine tissues to wipe the amniotic fluid, secretion, and blood from the surface of the pups' bodies.
6. Place the pups on a warming plate at 37℃, and softly pinch the tail of each pup a number of times with a pair of forceps until they start to breathe and turn sufficiently pink.

[From the Removal of the Uteruses through to the First Breaths of the Pups]
No. 19-01

Fostering

Select a foster mother whose pups have a different coat color than the caesarean sectioned pups, so that you can distinguish between them later.

1. Remove the foster mother from the cage.
2. Reduce the foster mother's litter of pups by half (for example, if the litter size of the foster mother is 10, remove 4-5 pups).
3. Mix the caesarean sectioned pups to be fostered (the same number of pups as the number of pups taken away) with the bedding, then mingle them with the remaining pups of the foster mother.
4. Put the foster mother back with the pups in the original cage.

[Fostering] No. 19-02

References

1. Nagy A., Gertsenstein M., Vintersten K., and Behringer R. 2003. Manipulating the Mouse Embryo, A Laboratory Manual (Third edition). *Cold Spring Harbor Laboratory Press.* ISBN 0-87969-591-9.