

# Embryo Manipulation after Mid-Gestation Stages in Mice

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**T**he system for directly manipulating mammalian embryos is an advantage not only to revealing the various developmental evidences but also in evaluating the availability of possible techniques for fetal surgery. "Exo utero development in mice" was reported by Muneoka et. al.<sup>1</sup> and has been one of the most useful techniques in experimental embryology<sup>2</sup>, including developmental neurobiology<sup>3</sup> and prenatal plastic surgery for malformations.<sup>4</sup> We have applied this method to establish experimental animal models with congenital disorders<sup>3,5</sup> and examined the mechanisms of normal and abnormal morphogenesis. In this paper, we would like to introduce the technique of exo utero development in combination with a microinjection system in mice embryos.

## EXPERIMENTAL PROCEDURE OF EXO UTERO DEVELOPMENT IN MICE

### Animal preparation

Animals used were Jcl:ICR (MCH) mice. A female (8–20 weeks old) was housed overnight with a male in the same cage. A vaginal plug was found the following morning; the day was designated embryonic day 0 (E0). Embryos can develop exo utero from E10; however, viability of embryos with manipulation after E11 is much higher, and therefore, we usually manipulate embryos after E11 (Table 1).

### Exo utero development in combination with microinjection

(1) Relax the myometrium with intraperitoneal injection (IP) of 1.4 mg of ritodrin hydrochloride per animal.

(2) Anesthetize dams with IP of 70 mg / kg body wt. of pentobarbital.

(3) Shave the abdominal hair, hold animals on the plate with tape, and sterilize the abdominal skin with 70 percent ethanol. We use the aluminum or stainless plates for the operating plates because they are heat-resistant during the process of fire sterilization or autoclaves.

(4) Incise the abdominal skin longitudinally from beneath the xiphoid process to the lower abdomen. The length of incision is usually up to 3 cm. Then detach the skin from the abdominal muscles. In this procedure, it is necessary to avoid injury of the superficial large vessels.

(5) Make a longitudinal incision of the anterior abdominal muscles just beside (left or right) the linea alba.

#### Procedure under the operating microscope

(6) Pull the right (or left) uterus (Figure 1a) and make small incision on the top of the uterus.

(7) Make longitudinal incision on the myometrium at the counterpart of the placenta (Figure 1b)

(8) With scissors cut the umbilical cord of unnecessary embryos and remove the embryos from the placen-

ta. Scissors usually causes only a small amount of bleeding; however, using sharpened forceps causes severe bleeding (i). The placenta must be left on the uterus without any injuries, because it has a well-developed vascular system. We usually leave 3 or 4 embryos in each horn of the uterus for manipulation.

(9) Remove the decidua gently to confirm that the embryos are under the operating microscope (Figure 2a) and that some solution, such as peptide, antibody, exogenous gene, suspended micro beads or cells, was injected into the embryos. We usually add a small amount of carbon ink (less than 1:500) into the injected solution to confirm injections (Figure 2b). We have found that this carbon ink has no harmful effects on our experiments.

(10) After manipulation, embryos are returned to the abdominal cavity of the dams.

(11) Repeat the same procedure on the another side of the uterus (ii).

#### Procedure with the naked eye

(12) Incision on the abdominal muscle is closed with 3- or 4-0 silk line.

(13) Before the last knot, the abdominal cavity of the dam is filled with 1.5 ml of Hank's solution (37°C) (iii).

(14) Incision of the abdominal skin is closed by autosuture (MikRon 9 mm Autoclip Applier).

(15) Finish surgical procedure (iv) and dams are placed on the hot plate at 37°C.

Comparison of *exo* - and *in-utero* development in mice embryos

Group	Number embryos	Viability (%)	Crown-rump** lenght (mm)	Body weight** (g)
E15 - E18 <i>exo-utero</i>	5	5 (100)	25.96 ± 0.619	1.56 ± 0.068
E14 - E18 <i>exo utero</i>	10	9 (90)	23.24 ± 2.549	1.33 ± 0.180
<i>in utero</i>	10	10 (100)	24.62 ± 2.356	1.35 ± 0.228
E12 - E18 <i>exo utero</i>	5	4 (80)	25.88 ± 1.924	1.80 ± 0.079
<i>in utero</i>	5	5 (100)	26.50 ± 2.070	1.74 ± 0.133
E13 - E15 <i>exo utero</i>	5	4 (100)	15.90 ± 0.523	0.49 ± 0.014
<i>in utero</i>	5	5 (100)	14.92 ± 0.950	0.47 ± 0.058
E12 - E15 <i>exo utero</i>	20	17 (85)	14.47 ± 0.964	0.44 ± 0.040
E11 - E15 <i>exo utero</i>	9	7 (78)	15.49 ± 0.701	0.50 ± 0.015

\* the day of surgery - the day of sacrifice

\*\* mean ± standard deviation

Table 1.

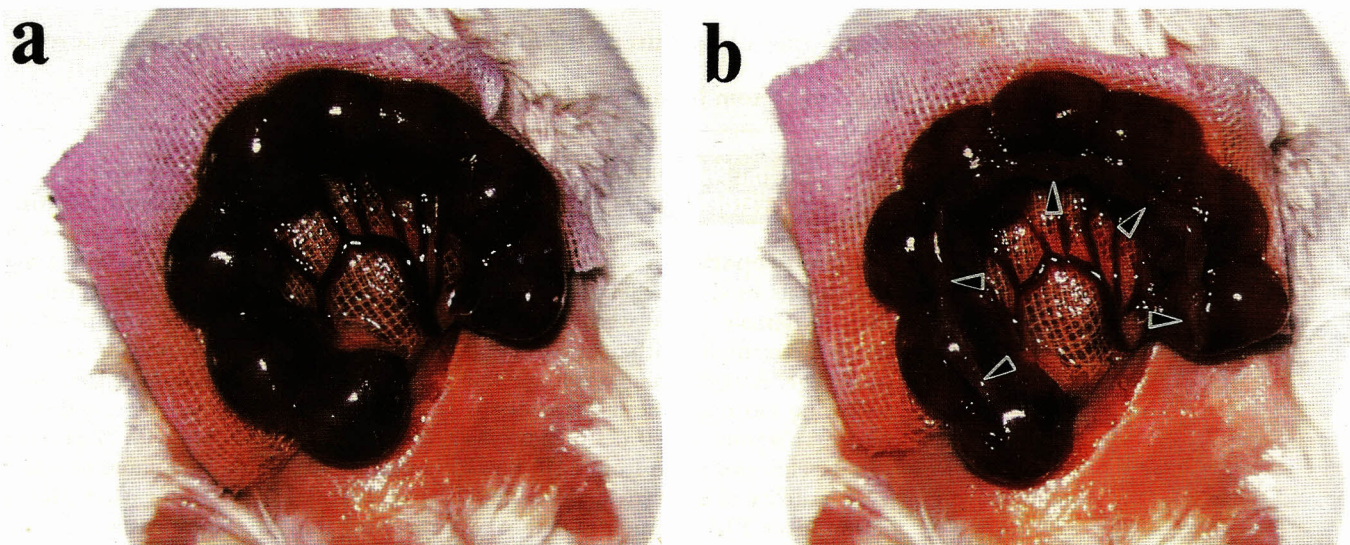


Figure 1. Incision of the myometrium. a) The left horn of uterus without incision on E13. We usually use the sterilized wet gauze with Hank's solution to avoid embryos drying. b) The incised horn of uterus. Usually 5 - 8 embryos in each horn are found. Arrowheads indicate the retracting myometrium.



## NOTES

(i) in case of severe bleeding from the umbilical cord, a small amount of fibrin paste is available to stop bleeding.

(ii) it is not necessary to close the incised uterus.

(iii) Hank's solution is sterilized by a syringe filter (pore size: 0.2  $\mu\text{m}$ ) and we don't use any antibiotics.

(iv) it is better to complete surgical procedure mentioned above (step 4 to 14) within 30 to 40 minutes.

### Microinjection system

Hard-quality glass tubes (Gl. 2, Narishige Scientific Instrument Lab, Tokyo, Japan) are pulled by a micro-electrode puller (PN-3, Narishige Scientific Instrument Lab, Tokyo, Japan), and the tip of the glass tubes are sharpened by a pipette grinder (MCG-II, Chatani Limited, Tokyo, Japan) and a micro forge (MF-79, Narishige Scientific Instrument Lab, Tokyo, Japan). The top of the pipettes

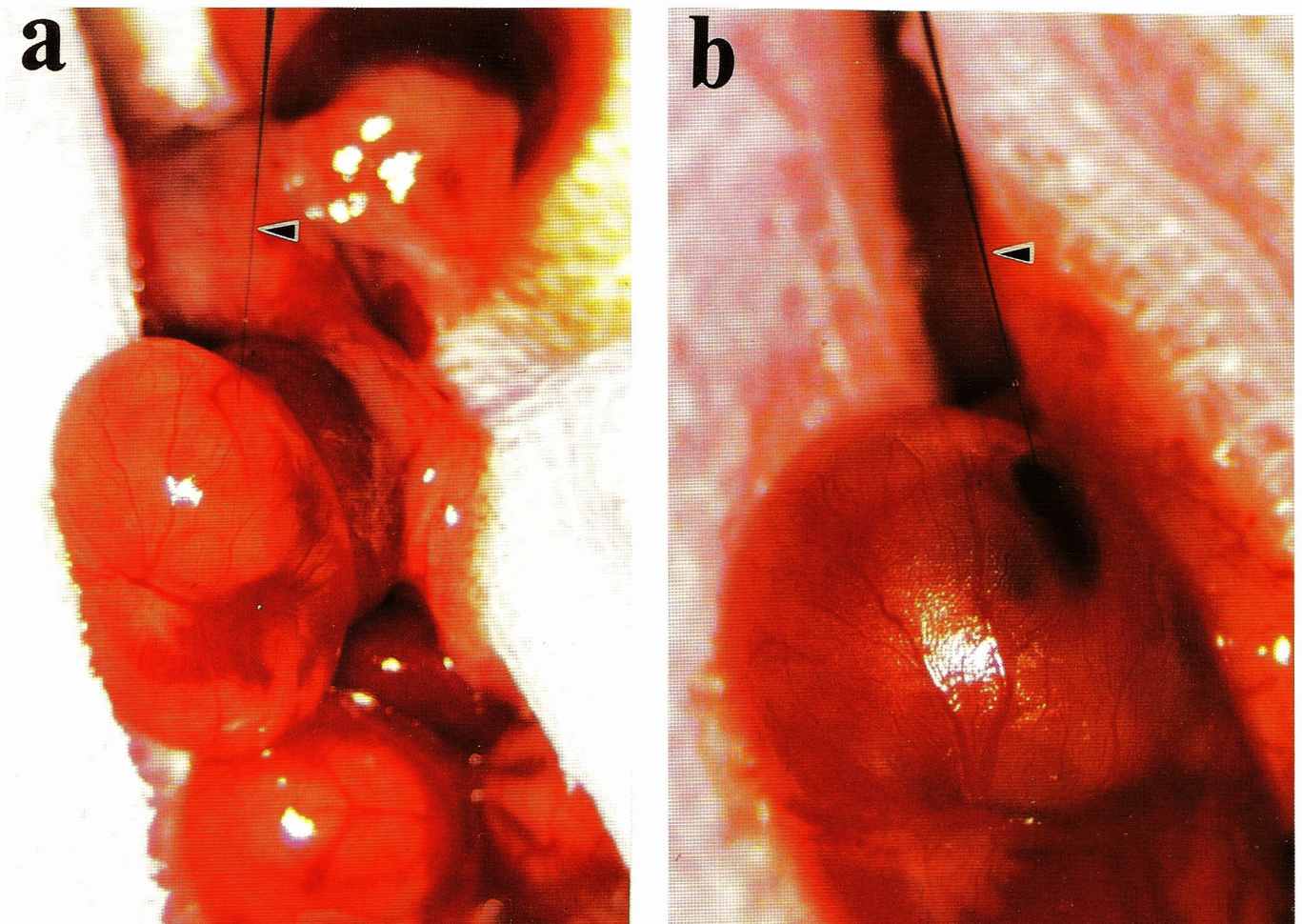
are usually within 10 to 50  $\mu\text{m}$  in diameter. Viability of embryos in this experiment mainly depends on the sharpness of the tip of the pipettes. Micropipettes are connected with Hamilton threaded plunger syringe (0.5 ml, #8100, USA) by a polyethylene tube. Without adding any air bubbles, the microsyringe, connecting tube, and micropipette must be filled with sterilized paraffin oil before sucking the injected solution.

### Application of exo utero development

Viability of embryos developed exo utero is high enough after E11 (Table 1); however, it may not be high enough at earlier stages than E11. One of the advantages of exo utero development is the possibility of manipulating specific regions under direct observation by an operating microscope. At earlier stages (before E11), the decidua is so thick that it is necessary to remove or cut the decidua to confirm details.

Before E11, the placenta has poorly developed. Nutrition, or essential substances, for developing embryos are provided by the decidua. Indeed, at earlier stages than E11, the decidua has a well-developed vascular system; therefore, incision of the decidua causes severe bleeding and may cause embryonic death.

In vertebrates, it has been possible to manipulate fish, amphibian and avian embryos directly after mid-gestation stages, but has been very difficult in mammals. However, since exo utero development was reported by Muneoka et. al.,<sup>1</sup> it has been easy to manipulate rodent embryos after mid-gestation stages, and it is possible to examine the effects of manipulation directly on histo- and/or organogenesis at term or even after birth (including adulthood) if we use foster mothers. In this respect, we are at a better advantage to manipulate mammalian embryos than other in vitro systems. **STI**



**Figure 2.** The injection of solution with carbon ink into the left telencephalic vesicle of E13 embryo. a) Details of embryos are clearly visible through the embryonic membrane with operating microscope. The decidua has already removed. b) The injection was clearly confirmed by the black color of carbon ink. Arrowheads indicate the micropipette.

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