Review

Fetal Laser Surgery *Exo Utero* in Mice*

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ABSTRACT It has been very difficult to investigate mammalian embryogenesis. Whole-
embryo culture systems allow the observation of mammalian embryogenesis and enable
the manipulation of the embryos, but it is almost impossible to culture fetuses at the late
gestational period. The *exo utero* method does allow the manipulation of mammalian
fetuses at the late gestational period. In this review, the detailed method of *exo utero*
surgery in mice is described, and the advantages and disadvantages of the applications of
laser equipment for fetal surgery are discussed.

It has long been known that arhinencephaly is often associated with agenesis of the
corpus callosum. The relationship between agenesis of the olfactory bulb and that of
the corpus callosum is not fully understood. In this review, an example of fetal laser surgery
*exo utero* was introduced, in which fissuration of the corpus callosum was induced after
the destruction of the anlage of the olfactory bulbs in the mouse embryo. It was thus
elucidated that agenesis of the olfactory bulbs induced the agenesis of the corpus callo-
sum.

Key words: laser, fetal surgery, *exo utero*, *Pdn*, arhinencephaly

It has been very difficult to investigate mammalian embryogenesis and manipulate the embryos, since they
develop in the uterus of the mother. A whole-embryo culture system which was developed mainly by New
and his colleagues (New et al., 1973; Cockcroft, 1973) allowed us to observe mammalian normal and abnor-
mal embryogenesis and manipulate the embryos. However, this whole-embryo culture system has a limited
culture period (New, 1978). It is almost impossible to culture fetuses at the late gestational period, and thus
*exo utero* surgery, which allows the manipulation of mammalian fetuses at the late gestational period, has
been developed (Muneoka et al., 1986; Wanek et al., 1989; Naruse and Kameyama, 1989, 1990; Naruse and
Tsutsui, 1989; Naruse and Keino, 1993; Naruse et al., 1995).

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severe cerebral malformations that are associated with agenesis of the corpus callosum are included in the cyclopia-arhinencephaly series (Warkany, 1971). Homozygotes of Polydactyl Nagoya mice (Pdn/Pdn) which have been inbred in our laboratory also show agenesis of the olfactory bulbs and agenesis of the corpus callosum (Naruse et al., 1990). The relationship between agenesis of the olfactory bulb and that of the corpus callosum is not yet fully understood (Schaefer et al., 1991).

In this review, we present an example of fetal laser surgery exo utero which was applied to induce arhinencephaly in mice, and to investigate the relationship between agenesis of the olfactory bulb and that of the corpus callosum. And it was elucidated that agenesis of the olfactory bulbs induced agenesis of the corpus callosum using fetal surgery exo utero (Naruse and Keino, 1993; Naruse et al., 1995).

The detailed method of fetal surgery exo utero surgery in mice is described, and the advantages and disadvantages of fetal laser surgery are discussed.

**MATERIALS AND METHODS**

The present experiment was done under the Guideline for Animal Experimentation, Institute for Developmental Research, Aichi Human Service Center.

1. Slc:ICR mice were obtained from Japan Slc. Inc., Shizuoka, Japan. Pregnancy was established by housing 6 to 8 females overnight with one male. Pregnancy was dated as day 0 if a vaginal plug was found in the morning.

2. Pregnant mice on day 13 of gestation were anesthetized with an intraperitoneal injection of 0.04 mg/g (body weight) of pentobarbital sodium (Nembutal Injection, Dai-Nippon Pharmaceuticals, Osaka, Japan). For the accurate dose of pentobarbital sodium, we diluted Nembutal Injection solution with 9 parts of sterile distilled water. Then, we injected 0.008 ml/g (body weight) of the diluted Nembutal solution. When anesthesia with Nembutal did not suffice, the mother mouse was temporarily supplemented by inhalation anesthesia with diethyl ether.

3. The mother mouse was injected intraperitoneally with ritodrine hydrochloride (1.4 mg/mouse, 0.12 ml of Utemerin Injection, Kissei Pharmaceuticals, Matsumoto, Japan) to relax the mother’s uterus.

4. The mother mouse was then injected intraperitoneally with 0.025 ml/mouse of Antibiotic-Antimycotic 100X solution (penicillin G sodium, streptomycin sulfate, and amphotericin B mixture; GIBCO BRL, New York, USA). For the accurate dose, we diluted Antibiotic-Antimycotic 100X solution with 9 parts of sterile distilled water. Then, we injected 0.25 ml/mouse of the diluted solution.

5. The mother mouse was settled on her back on a cork board.

6. Before the operation, the abdomen of the mouse was wetted with 70% alcohol to disinfect and then with warm (37°C) Hanks’ balanced salt solution (GIBCO BRL).

7. An incision about 1 cm long was made longitudinally in the midline of the abdominal skin of the mother. Then, another incision of the same length was made longitudinally in the abdominal muscle.

8. The uterus was displaced from the mother’s abdominal cavity through the incision in the abdominal skin and muscle.

9. Usually 2 embryos near the ovary in each uterine horn received fetal laser surgery. The remaining embryos were removed by suction with a syringe with an 18G needle and discarded to provide more room for the fetuses which received surgical treatment.

10. A small incision (about 1 mm) was made in the uterine myometrium at a position opposite the placenta
Fetal Laser Surgery _Exo Utero_ in Mice

109

11. An argon laser beam (Nidek AC-2000, wavelength 488.0 and 514.5 nm, Nidek, Gamagori, Japan), guided by a flexible quartz light fiber rod, was delivered from a hand-piece which was especially manufactured for this operation by Nidek. An aiming laser beam was focused on the anlage of the olfactory bulb in the mouse embryo on day 13 of gestation using a laser goggle under a dissection microscope, and a laser beam (2 watts, 0.3 seconds) was shot by a foot switch through the yolk sac membrane and amnion (Fig. 2). We could see just the focused spot (φ25 μm) of the aiming laser beam on the anlage of the olfactory bulb using the laser goggle. Thus, we induced fetal tissue destruction (Fig. 3A) without damaging the yolk sac membrane and amnion, and without leakage of amniotic and extra-embryonic fluid.

12. The uterus and conceptuses, which received laser surgery, were replaced into the mother’s abdominal

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Fig. 1  Fetal laser surgery on day 13 of gestation _exu utero_ in mice. A small incision was made on the uterine myometrium at a position opposite the placenta. Thus, the conceptuses were taken out of uterus but still kept in contact with the uterine epithelium via the placenta. The argon laser beam from a hand-piece was irradiated to destroy the anlage of the olfactory bulbs through the yolk sac membrane and amnion. (Neurosci. Protocols, 3: 2, 1-11, 1995)
cavity without suturing the myometrium. The conceptuses were kept in contact with the uterus via the placent-
a.

13. The abdominal muscle was sutured by No. 6 silk suture thread, and 1–2 ml of warm (37°C) sterile
Hanks’ balanced salt solution was injected to fill the abdominal cavity of the mother. Then the abdominal
skin was sutured with No. 6 silk suture thread.

14. After surgery, the mouse was placed on a warm plate (Microwarm Plate MP10DM, Kitazato Supply,
Tokyo, Japan) at 37°C to prevent body temperature loss before the mouse regained consciousness.

15. On day 18 of gestation, the mother was killed by cervical dislocation and the surviving fetuses exo
uterum were observed grossly. Each brain was recovered from the cranium in Hanks’ balanced salt solution,
and fixed with Bouin’s solution.

16. The artificially induced arhinencephalic brain was embedded in paraffin. Frontal serial sections were
made at 10 μm thickness, and stained with hematoxylin and eosin.
Fetal Laser Surgery Exo Utero in Mice

Fig. 3  A: Mouse fetus on day 13 of gestation irradiated with laser beam in both anlagen of the olfactory bulbs (arrows) through the yolk sac membrane. To show the fetus clearly, the yolk sac membrane and amnion were removed.
B: Arhinencephalic mouse brain on day 18 induced by laser irradiation on day 13 of gestation. The olfactory bulbs are absent (arrows).
C: In the arhinencephalic brain induced by laser irradiation, the connection of corpus callosum fibers (CC) was blocked by the sulcus medianus telencephali medii (arrow). Bar = 1 mm. (Dev. Brain Res., 71: 69-74, 1993)

RESULTS AND DISCUSSION

On day 18 of gestation, the normal fetal mouse brain has 2 olfactory bulbs at the front of each cerebral hemisphere. It exhibits a well-developed corpus callosum and anterior commissure in the frontal histological sections. In the present study, in the arhinencephalic mouse brains of which the olfactory bulbs were de-
stroyed by fetal laser surgery on day 13 of gestation (Fig. 3B), the connection of corpus callosum fibers was blocked by the sulcus medianus telencephali medii (Fig. 3C). An abnormal hippocampal commissure, an irregular connection of anterior commissure, and abnormal formation of the thalamus were also observed.

In a mouse fetus which had lost its olfactory bulb on one side, the cerebral hemisphere on the same side shifted rostrally (Naruse and Keino, 1993). In light of this observation, we speculated that agenesis of the olfactory bulb had induced a shift of the telencephalon in order to fill the cranial space including the olfactory bulb area. In a mouse fetus which had lost its right olfactory bulb, the right cerebral hemisphere shifted rostrally to the level of the tip of the left olfactory bulb, and the connection of the corpus callosum fibers was also blocked (Naruse and Keino, 1993). Deformed massa commissuralis was observed in the brains whose olfactory bulbs were lost on day 15 of gestation (Naruse and Keino, 1993). The shift may have induced a geometric lag of both lamina reuniens in the cerebral hemispheres, and induced dysgenesis of the massa commissuralis, which is considered to be the bed for the decussation of corpus callosum fibers in the normal brain (Graumann, 1950; Rakic and Yakovlev, 1968). It was thus considered that the destruction of anlage of the olfactory bulbs induced the deformed massa commissuralis, and that the fissuration of the corpus callosum fibers was induced because of the deformed massa commissuralis (Naruse and Keino, 1993).

Fetal laser surgery has been ascertained to be useful also in sheep (DeVore et al., 1983) and chick (Been et al., 1981) embryos. We believe that fetal surgery like these experiments (Muneoka et al., 1986; Wanek et al., 1989; Naruse and Kameyama, 1989, 1990, Naruse and Keino, 1993; Naruse et al., 1995) will serve to elucidate the sequential manifestation of the combined abnormalities that have been explained as pleiotropism (Naruse and Keino, 1995).

To rear the fetuses which received fetal surgery, fetuses can be recovered from the mother’s abdominal cavity on day 18 of gestation and revived by incubation on a wet warm plate at 37°C. It is subsequently possible to rear them further using a foster mother which has just delivered (Naruse and Tsutsui, 1989).

The greatest advantage of fetal laser surgery is that particular tissues inside the other embryonic tissues can be destroyed without damage to the other tissues. Another advantage is no leakage of the extra-embryonic and amniotic fluid, which results in better postoperative circumstances for the fetuses than surgery using electrode or scissors. One disadvantage of fetal laser surgery is that the embryonic tissue cannot be manipulated in ways other than destroying it. A second disadvantage is that the degree of damage of the irradiated tissue cannot be precisely determined. A third disadvantage is that mouse embryos receive the laser beam on their mother’s abdomen, that is, missed-shot happens occasionally because of the mother’s respiratory movement.

It is very difficult to develop embryos exo utero after surgery before day 13 of gestation, i.e., the survival rate is very low. The yolk sac membrane becomes transparent due to the regression of the decidua from day 11.5 of gestation. Before day 11.5 of gestation, a window in the decidua on the yolk sac membrane must be opened. This manipulation causes too much disturbance of the fetal development. Accordingly, the survival rate after surgery with these early embryos is extremely low. After day 14 of gestation, fetal surgery exo utero becomes much easier, and the survival rate is more than 70%. From these knowledge, it was considered that day 13 of gestation may be the earliest stage of the mouse embryo to get consistent and reliable data. Cleft palate is occasionally manifested in the fetuses which received fetal surgery, though the reason why is unknown. It seems that there are strain differences in mice in the mortality rate after receiving fetal surgery.
REFERENCES


