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Fetal tracheal occlusion in mice: a novel transuterine method

Emrah Aydin, MD,[a,*] *Rashika Joshi, PhD,*[b] *Marc Oria, MD,*[a] *Brian Michael Varisco, MD,*[b] *Foong-Yen Lim, MD,*[a] *and Jose Luis Peiro, MD, PhD*[a]

[a] *The Center for Fetal, Cellular and Molecular Therapy, Cincinnati Fetal Center, Division of Pediatric General and Thoracic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*

[b] *The Division of Critical Care Medicine, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio*

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ABSTRACT

Background: Fetal tracheal occlusion (TO) is an emerging surgical therapy in congenital diaphragmatic hernia that improves the fetal lung growth. Different animal models of congenital diaphragmatic hernia and TO present advantages and disadvantages regarding ethical issues, cost, surgical difficulty, size, survival rates, and available genetic tools. We developed a minimally invasive murine transuterine TO model, which will be useful in defining how TO impacts lung molecular biology, cellular processes, and overall lung physiology.

Materials and methods: Time-mated C57BL/6 mice underwent laparotomy at embryonic day 16.5 (E16.5) with transuterine TO performed on two fetuses in each uterine horn. At E18.5, dams were sacrificed and fetuses harvested. The lungs of the TO fetuses were compared with the nonmanipulated counterparts by morphometric and histologic analysis.

Results: Successful TO was confirmed in 16 of 20 TO fetuses. Twelve of them survived to E18.5 (75%). Fetal weights were comparable, but lung weights were significantly greater in TO (28.41 ± 5.87 *versus* 23.38 ± 3.09, $P = 0.043$). Lung to body weight ratio was also greater (0.26 ± 0.003 *versus* 0.22 ± 0.002, $P = 0.006$). E18.5 TO lungs demonstrated dilated central and distal airspaces with increased cellularity. DNA/protein and DNA/lung weight ratios were elevated while protein/lung weight ratio was lower in TO compared to control.

Conclusions: Mice fetal transuterine TO is feasible with comparable outcomes to other current animal models. The increase in the lung weight, lung to body weight ratio and the DNA/protein ratio indicate organized lung growth rather than edema or cell hypertrophy.

Introduction

Fetal tracheal occlusion (TO) is an emerging prenatal therapy for severe congenital diaphragmatic hernia (CDH).[1] TO prevents the egress of pulmonary fluid, thereby increasing tissue stretch and accelerating lung growth, and may ameliorate the pulmonary hypoplasia of CDH. In animals and humans developing CDH, their lungs experience increased

* *Corresponding author*. The Center for Fetal, Cellular and Molecular Therapy, Cincinnati Fetal Center, Division of Pediatric General and Thoracic Surgery, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, MLC 11025, Cincinnati, OH 45229-3039, USA. Tel.: +1 513 803 4563; fax: +1 513 636 2735.
E-mail address: dremrahaydin@yahoo.com (E. Aydin).

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growth after fetal TO during the canalicular and saccular phases of their development.[2] The persistently high mortality of CDH over the past decades, despite advances in neonatal resuscitation and intensive care, increases the need for more investigation into the pathophysiology and prenatal treatment of the disease. To better understand how TO impacts human lung growth and development in CDH, mouse, rat, rabbit, and sheep models of TO have been developed.[1,3-5] Each model presents advantages and disadvantages regarding ethical issues; cost; surgical difficulty; animal, fetus, and lung size; survival rates; and available genetic tools.[4] However, each of these prior models requires hysterotomy for TO. Herein, we present an easy and minimally invasive murine TO model, which will be useful in defining how TO impacts in lung molecular biology, cellular processes, and overall lung physiology both in normal and CDH lungs.

Material and methods

Experimental design

Following approval of IACUC protocol #2016-0068 by the Cincinnati Children's Research Foundation Institutional Animal Care and Use Committee, age-matched wild-type C57BL/6 mice were mated, and the date when the vaginal plug is seen was accepted as embryonic day 0 (E0). At E16.5, pregnant dams underwent laparotomy and transuterine TO was performed upon two fetuses in each uterine horn with 2.5× magnification. At E18.5, all dams were harvested by cesarean section and fetuses were weighed by using a scale measuring accurately up to 0.001 g (Mettler Toledo, Columbus, OH). Lungs were dissected from mouse embryos and were weighed to calculate total lung to body weight ratio (LBWR). Normal fetuses of the litter served as nonmanipulated controls.

TO surgical method

After performing a median laparotomy to the dams under general anesthesia, the uterine horn was positioned gently in a transverse fashion with the pups facing upward between two fingers of the surgeon. Using gentle pressure, pups' heads were extended allowing visualization of the neck. A 6.0 polypropylene suture (13 mm 1/2c Taperpoint; Ethicon, Somerville, NJ) with an atraumatic needle was used for TO. The needle was inserted through the side of the uterus opposite to the placenta, through 1/3 anterior of the neck in a transverse fashion (Fig. 1A). The tip of the needle was gently advanced till it passes the midline of the pup where it was directed through the anterior of the neck and exits the neck between the

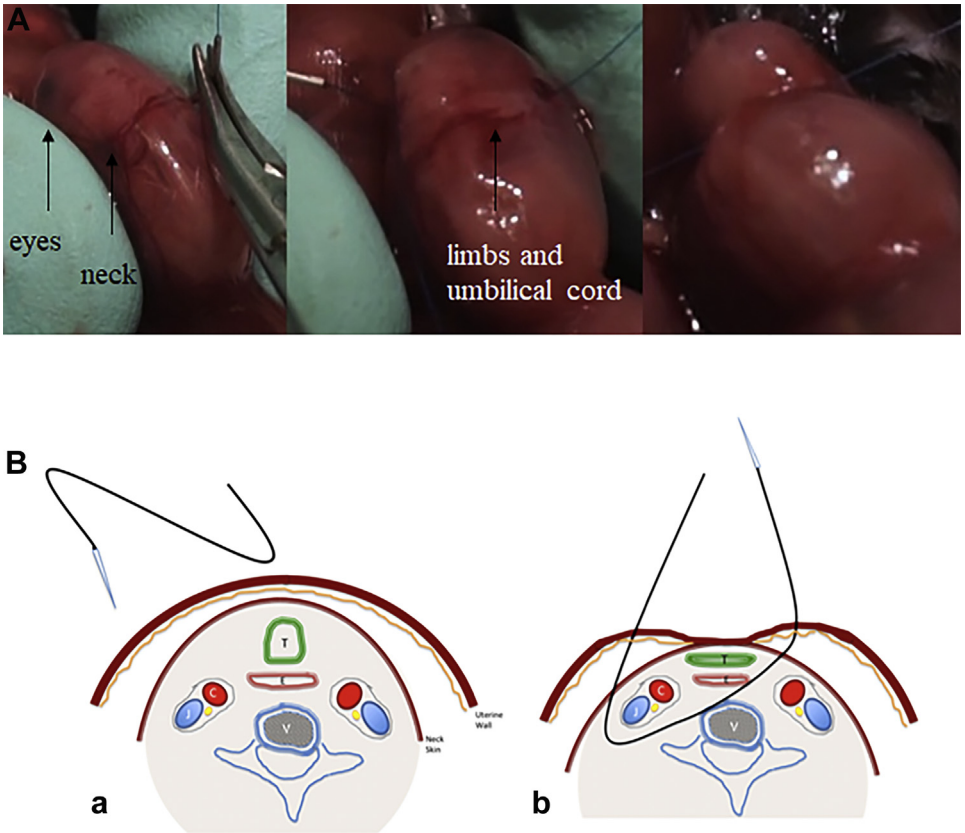

Fig. 1 – (A) The transuterine bypassing of the suture through the neck. (B) Schematic representation of the transuterine suture through the neck, respecting one side vascular (carotid and jugular) elements and surrounding trachea. (a) The entrance of the needle. (b) The structures included by the suture. C = carotid artery; J = jugular vein; T = trachea; E = esophagus; V = vertebra.