Journal of Pediatric Surgery (2006) 41, 1214–1218

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

# Hyperoncotic enhancement of fetal pulmonary growth after tracheal occlusion: an alveolar and capillary morphometric analysis☆

**Shaun M. Kunisaki, Robert W. Chang, Steven Andreoli, Sara Lewicke, Snow Peña-Peterson, Russell W. Jennings, Dario O. Fauza***

*Department of Surgery, Children's Hospital Boston, Boston, MA 02115, USA*
*Department of Surgery, Harvard Medical School, Boston, MA 02115, USA*

**Index words:**
Congenital diaphragmatic hernia;
Pulmonary hypoplasia;
Fetal tracheal occlusion;
Fetal surgery;
Fetus;
Lung development;
Fetal lung liquid;
Albumin;
Lung morphometrics

**Abstract**

**Background/Purpose:** Previous work has shown that intrapulmonary delivery of oncotic agents enhance overall lung growth after late gestational fetal tracheal occlusion (TO). This study was a post hoc analysis aimed at determining whether actual alveolar and capillary hyperplasias are maximized in this setting.

**Methods:** Twenty-one near term fetal lambs were evenly divided into 4 groups: group I comprised sham-operated controls; group II had TO alone; and groups III and IV underwent TO and intratracheal infusion of equal amounts of either saline or 25% albumin, respectively. Approximately 2 weeks thereafter, their lungs were examined by detailed alveolar and capillary morphometry before birth. Statistical analysis included analysis of variance and the Bonferroni correction for multiple comparisons ($P < .05$).

**Results:** Total alveolar and capillary numbers, as well as total alveolar surface area, were significantly higher in group IV and lower in group I compared with all other groups, with no differences between groups II and III. Alveolar capillary load was normal in all groups.

**Conclusions:** Intrapulmonary delivery of concentrated albumin safely enhances short-term alveolar and capillary hyperplasia in a late gestational model of fetal TO. This therapeutic concept may allow for TO to be effective and predictable when performed late in gestation.



Clinical experience with fetal tracheal occlusion (TO) for treatment of pulmonary hypoplasia associated with congenital diaphragmatic hernia (CDH) has not improved survival, when prospectively compared with state-of-the-art postnatal therapy [1]. Despite developments in minimally invasive techniques for TO, preterm labor continues to be a major limiting factor, responsible, to a great extent, for such disappointing results [1,2]. Attempts at performing TO late in gestation, to avoid prematurity, have not resulted in fetal lung growth because of the physiological decrease in fetal lung liquid secretion observed before birth [2-4] (Deprest J,

☆ SMK was supported by grants from the NIH National Research Service Award DK065406-02 and the VH Kazanjian Surgical Research Fellowship of the Massachusetts General Hospital.

* Corresponding author. Children's Hospital Boston, Boston, MA 02115, USA. Tel.: +1 617 919 2966; fax: +1 617 730 0910.
*E-mail address:* dario.fauza@childrens.harvard.edu (D.O. Fauza).

0022-3468/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2006.03.001

personal communication, 2004). Another limitation of simple TO is the passive nature of the method because it depends on spontaneous fetal lung liquid secretion postoperatively, which can be erratic and unpredictable. This may lead to exceedingly inconsistent pulmonary growth responses, which can vary from no response at all to overwhelming lung hyperplasia and mediastinal compression resulting in hydrops and fetal demise [1,5-8].

We have previously shown, experimentally, that delivery of a hyperoncotic colloid agent to the fetal lung liquid enhances overall pulmonary growth after TO performed late in gestation [9,10]. Should this strategy maximize actual pulmonary hyperplasia after TO, it could allow for this intervention to be effective even when performed near term, thus avoiding prematurity. It might also minimize the variability in the pulmonary growth response because of the active expansion of fetal lung liquid volume induced by local positive oncotic pressure. This study was aimed at determining whether this concept enhances actual alveolar and capillary hyperplasia after TO.

## 1. Materials and methods

The Harvard Medical School animal management program is sanctioned by the American Association for the Accreditation of Laboratory Animal Care (file no. 000009) and meets National Institutes of Health Standards as set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council Publication, revised 1996). The present study was approved by the Harvard Medical School Standing Committee on Animals, under protocol no. 03355.

This was a post hoc alveolar and capillary morphometric analysis on animals of a previously reported study from our group [10].

### 1.1. Fetal surgical manipulation and delivery

Time-dated pregnant ewes at 120 to 129 days of gestation (mean, 123 $\pm$ 2.6 days; term = 145 days) were anesthetized with 2% to 4% halothane (Halocarbon Laboratories, River Edge, NJ) and received 1000 mg of cefazolin (BMH, Philadelphia, Pa) intravenously before surgical manipulation. The bicornuate uterus was exposed through a midline laparotomy. Fetal lambs (n = 21) were divided into four groups: group I (n = 5) comprised sham-operated controls; group II (n = 5) underwent simple TO as previously described [11]; group III (n = 5) had TO followed by injection of 60 mL of normal saline into the distal trachea; and group IV (n = 6) underwent TO plus intratracheal infusion of 60 mL of 25% human albumin (Buminate, Baxter Healthcare, Deerfield, Ill). The amniotic fluid, which had been previously removed and kept at 37°C, was reinfused into the amniotic cavity, together with 500 mg of cefazolin, at the end of the fetal procedure. The gestational membranes and uterine wall were closed in one layer with a TA 90-mm titanium surgical stapler (United States Surgical Corp, Norwalk, Conn). The maternal abdomen was closed in layers. On the first postoperative day, all ewes received 1.2 million units of benzathine penicillin intramuscularly (Wyeth Laboratories, Philadelphia, Pa). Fetuses were delivered by cesarean section near full term, 15 to 18 days postoperatively (mean, 15.9 $\pm$ 1 days), immediately after maternal euthanasia with a lethal dose of Somlethal (JA Webster, Sterling, Mass). There was no difference in the duration of the postoperative period among the groups by repeated measures analysis of variance.

### 1.2. Lung preparation and analysis

Each lamb was weighed and then had the chest opened through a median sternotomy. Lung liquid was aspirated through the trachea and analyzed for osmolarity, pH, and electrolyte content on a Stat Profile Ultra blood gas and electrolyte analyzer (Nova Biomedical Corp, Waltham, Mass). Lung liquid albumin levels were measured using established techniques [12,13]. The trachea and both lungs were then removed en bloc and inflated with saline at 20 cm $H_2O$ pressure. Lung volumes were determined by water displacement of the inflated lungs. To standardize lung volumes for differences in body weight, lung volume to body weight (LV/BW) ratios were calculated.

Samples of lung tissue were then taken from standard positions at the periphery of the right and left apical and diaphragmatic lobes, fixed in 10% neutral-buffered formalin (Sigma, St Louis, Mo) and paraffin embedded. Sections were stained with H&E for alveolar morphometric analysis. Additional sections underwent immunohistochemical staining with a von Willebrand factor antibody (Dako Corp, Glostrup, Denmark), at 1:400 dilution, to evaluate the fetal pulmonary vascular endothelium, as described by Morrell et al [14]. Secondary antibody detection was by a multispecies link ultra-streptavidin detection system (Signet Laboratories, Dedham, Mass), according to the manufacturer's instructions.

Intraacinar alveolar and capillary morphometric analysis was performed by two blinded investigators using a Zeiss laboratory microscope (Carl Zeiss, Jena, Germany) equipped with an eyepiece engraved with a multipoint coherent test lattice, at a magnification of 200 times. Airspace fraction, total alveolar number, and total alveolar surface area were determined by the method of Weibel and Gomez, as modified by Dunnill [15]. Total alveolar capillary number was calculated as previously described [16]. Alveolar capillaries were defined as von Willebrand factor–positive structures less than 25 $\mu$m in diameter directly adjacent to a gas exchange surface. For all measurements, at least three random sections per lobe were analyzed, resulting in a total of at least twelve lung sections per animal. Three to four magnified fields per section were examined.

Lung tissue samples were also fixed and embedded for transmission electron microscopy (TEM) as previously described [17]. Silver sections were cut with a LKB