0031-3998/04/5605-0818
PEDIATRIC RESEARCH
Vol. 56, No. 5, 2004

Infant Pulmonary Function in a Randomized Trial of Fetal Tracheal Occlusion for Severe Congenital Diaphragmatic Hernia

ROBERTA L. KELLER, SAMUEL HAWGOOD, JOHN M. NEUHAUS, DIANA L. FARMER, HANMIN LEE, CRAIG T. ALBANESE, MICHAEL R. HARRISON, AND JOSEPH A. KITTERMAN

The Cardiovascular Research Institute and Department of Pediatrics [R.L.K., S.H., J.A.K.], Department of Epidemiology and Biostatistics [J.M.N.], and Department of Surgery [D.L.F., H.L., C.T.A., M.R.H.], University of California San Francisco, San Francisco, CA 94143

ABSTRACT

Congenital diaphragmatic hernia (CDH) carries a high mortality risk secondary to pulmonary hypoplasia and respiratory failure. In experimental animals, fetal tracheal occlusion (TO) induces lung growth and morphologic maturation. We measured indicators of pulmonary function in 20 infants who were enrolled in a randomized trial of fetal TO as treatment for severe CDH [nine with conventional treatment (controls); 11 with TO]. We hypothesized that TO would improve lung function. At birth, the TO group had a lower mean gestational age (30.8 ± 2.0 *versus* 37.4 ± 1.0 wk; $p = 0.0002$). All infants required assisted ventilation. Mortality did not differ between groups (64 *versus* 78%, TO and control, respectively; $p = 0.64$). We measured respiratory mechanics at four study points: *1*) first 24 h, *2*) before CDH operative repair (5.9 ± 2.2 d), *3*) immediately after repair (7.0 ± 2.2d), and *4*) before elective extubation (32.5 ± 16.1 d). We calculated perioperative oxygenation index and alveolar-arterial oxygen difference to assess efficiency of pulmonary gas exchange. Data were analyzed by univariate and repeated measures techniques. Respiratory system compliance (Crs) was low. The rate of increase in Crs over the four study points was greater in the TO group than in control subjects. Crs in the TO group was significantly greater at study 2 (0.28 ± 0.12 *versus* 0.17 ± 0.04 $mL \cdot cm\ H_2O^{-1} \cdot kg^{-1}$; $p = 0.02$) and study 4 (0.93 ± 0.45 *versus* 0.51 ± 0.16 $mL \cdot cm\ H_2O^{-1} \cdot kg^{-1}$; $p = 0.02$). oxygenation index did not differ between groups, but alveolar-arterial oxygen difference was lower in the TO infants. We conclude that fetal TO for severe CDH results in modest improvements in neonatal pulmonary function that are of questionable clinical significance. (***Pediatr Res* 56: 818–825, 2004**)

Abbreviations

$AaDo_2$, alveolar-arterial oxygen difference
BMZ, betamethasone
CDH, congenital diaphragmatic hernia
Crs, compliance of respiratory system
ECMO, extracorporeal membrane oxygenation
FIO_2, fraction of inspired oxygen
GA, gestational age
HFV, high-frequency ventilation
iNO, inhaled nitric oxide
LHR, lung-to-head ratio
OI, oxygenation index
$PaCO_2$, arterial carbon dioxide pressure
PaO_2, arterial oxygen pressure
P_{aw}, mean airway pressure
PEEP, positive end expiratory pressure
PEF, peak expiratory flow
SPO_2, arterial oxygen saturation measured by pulse oximetry
TO, tracheal occlusion
V_E, minute ventilation

Congenital diaphragmatic hernia (CDH) occurs 1 in 3000 live births (1). A defect in formation of the hemidiaphragm results in fetal and neonatal pulmonary parenchymal and vascular hypoplasia (2–7). This is further complicated by a reactive pulmonary vascular bed that is poorly responsive to vasodilator therapy (8–11). As a result, newborns with CDH may require prolonged intensive support with oxygen, assisted ventilation, and extracorporeal membrane oxygenation (ECMO) to maintain adequate ventilation and oxygenation (6,12–14). Individual centers have reported recent improvements in survival rates to >75% for all newborns who are admitted with CDH (15–17). However, other studies that included multiple centers have reported that mortality from CDH has remained high (45%) for liveborn infants (18) and those who are admitted to

Received November 19, 2003; accepted June 15, 2004.
Correspondence: Roberta L. Keller, M.D., 533 Parnassus Avenue, UCSF Box 0748, San Francisco, CA 94143; e-mail: rkeller@itsa.ucsf.edu
R.L.K. is a recipient of the Glaser Pediatric Research Network Award. This study was supported by National Institutes of Health grant HL62433 and the Pediatric Clinical Research Center, Moffitt Hospital, University of California, San Francisco, with funds provided by the National Center for Research Resources, 5 M01 RR-01271.

DOI: 10.1203/01.PDR.0000141518.19721.D7

a surgical center (1,19), despite advances in neonatal and surgical care. Population-based studies have shown that infants whose CDH is diagnosed antenatally are at highest risk for neonatal death (50–65%) (1,18).

Previous studies of infants with CDH have shown that compliance is low and may decrease acutely after surgical repair of the hernia (20,21). With delayed hernia repair, compliance increases from birth to time of repair (22). Some investigators have found that higher preoperative compliance is associated with improved outcome, including survival (21–24). With CDH, efficiency of gas exchange is poor with respect to both ventilation and oxygenation, and the severity of impairment of gas exchange in the newborn period has also been associated with differences in survival (5,14,23). Right-to-left shunting away from a high-resistance pulmonary vascular bed through the foramen ovale or a patent ductus arteriosus results in hypoxemia. This shunt progressively decreases in infants who survive (25,26).

Because of the high mortality associated with CDH, temporary fetal tracheal occlusion (TO) was developed for high-risk fetuses to attempt to enhance fetal lung growth and improve postnatal lung function. Experimental animal models of CDH and fetal TO in rodents, rabbits, and sheep have demonstrated increased fetal lung growth and morphologic maturation, and normalization of the pulmonary vasculature (27–32), although there is evidence of a deleterious effect of TO on alveolar type II cell number and function (33–35). In fetal sheep with CDH, variable effects on gas exchange have been seen after fetal TO over several hours of postnatal stabilization, with some investigators demonstrating improvements (28,36) and others finding no differences (37). We undertook a randomized, controlled trial of endoscopic TO in fetuses with severe, isolated left CDH to compare survival at 90 d with fetal intervention with that with standard care (38). Twenty-four fetuses were enrolled in the trial; 20 were born at our center (nine control subjects with standard care, 11 with TO). To evaluate lung function in these 20 infants who were receiving mechanical ventilation, we performed pulmonary function tests with an in-line pneumotachometer at four critical time points: *1*) in the first 24 h after birth, *2*) within 24 h before CDH operative repair, *3*) within 24 h after CDH repair, and *4*) before elective tracheal extubation. In addition, we evaluated efficiency of gas exchange during the perioperative period. We hypothesized that TO for severe left CDH would improve lung function in the newborn period. Secondarily, we hypothesized that lung function would be better in patients who ultimately survived and would be worse in patients in the most severe group (defined by prenatal measures).

METHODS

This study was approved by the University of California San Francisco (UCSF) Committee on Human Research. Written, informed consent was obtained before enrollment of fetal subjects. All studies took place in the UCSF Intensive Care Nursery. Subjects were born from July 1999 through August 2001.

Randomized trial: inclusion/exclusion criteria, fetal intervention, and antenatal care. The detailed trial protocol and outcome have been described previously (38). Briefly, fetuses were evaluated at UCSF at 22–27 wk gestational age (GA). Fetal inclusion criteria for the trial included ultrasound diagnosis of a severe, isolated left CDH [defined as liver herniated into the thorax, lung-to-head ratio (LHR) <1.4, no associated anomalies, normal fetal karyotype, and normal fetal echocardiogram]. LHR has previously been shown to be a powerful predictor of mortality in this population (39). Fetal endoscopic TO was accomplished at 24–27 wk GA with either an external tracheal clip (40) (two patients) or a detachable silicone balloon (Boston Scientific/Target Therapeutics, Natick, MA) that was inflated with isosmotic contrast material (41) (nine patients).

All mothers who underwent fetal intervention were treated perioperatively with a standardized protocol of tocolysis. A single maternal glucocorticoid dose [betamethasone (BMZ) 12 mg i.m.] was given before TO to attempt to enhance the effect of the intervention on fetal lung growth (42). A course of maternal BMZ to accelerate fetal lung maturation was administered when preterm delivery ($\leq$34 wk) was thought to be imminent. Delivery of fetuses after TO was determined by concerns for fetal or maternal well-being. This resulted in preterm delivery of all newborns in the TO group (Table 1). Newborns who underwent TO were delivered by cesarean section (*ex utero* intrapartum therapy procedure (43)), which allowed evaluation of the fetal airway, release of TO, endotracheal intubation, administration of prophylactic exogenous surfactant (Exosurf Neonatal 3 mL/kg; Glaxo Wellcome, Research Triangle, NC), and initiation of pulmonary ventilation while the fetus was maintained on umbilical placental circulation.

Control infants were delivered vaginally after labor at $\geq$36 wk GA. An antepartum course of maternal BMZ was administered when the amniotic fluid lung profile was immature (Table 1). The trachea was intubated immediately after birth, and prophylactic surfactant was administered in most of the infants to attempt to standardize care between the two groups (Table 1).

Study population. Lung function data were collected on the 20 newborns who were delivered at UCSF (nine control subject, 11 TO). As noted, all newborns in the TO group were preterm ($\leq$36 wk GA), with significantly lower GA and birth weight than control subjects (Table 1). Mean LHR of control subjects and those who underwent TO were not significantly different (Table 1). All infants had severe diaphragmatic defects that required prosthetic patch repair. There were no differences in age at CDH repair or age at elective tracheal extubation. Survival was 70% (14 of 20), with no difference between groups (Table 1). Of the infants who had measurements of pulmonary mechanics, seven of 20 (35%) had an LHR $\leq$0.90 [most severe CDH (38)].

Neonatal respiratory care. Conventional mechanical ventilation was the ventilatory mode of choice, with ventilator rates up to 90 breaths per minute, short inspiratory times, and low positive end expiratory pressure (PEEP; 2–3 cm H_2O). Inspiratory pressure was set to achieve stable, acceptable oxygenation.

Table 1. *Clinical characteristics and outcomes of 20 infants with severe CGH treated by fetal TO or conventional perinatal care*

	Control (n = 9)	TO (n = 11)	p value
Male sex	6 (67%)	8 (73%)	0.58
LHR	0.95 ± 0.14 (0.80–1.20)	0.97 ± 0.21 (0.50–1.30)	0.53
Duration of TO (d)	N/A	36.2 ± 14.7 (16–64)	
GA (wk)	37.4 ± 1.0 (36–39)	30.8 ± 2.0 (28–34)	0.0002
Birth weight (kg)	3.17 ± 0.41	1.49 ± 0.36	<0.0001
Antenatal glucocorticoids	7 (78%)	11	0.19
Surfactant treatment	5 (56%)	11	0.03
Repeat dose(s)	0	3 (27%)	0.22
Perioperative iNO	5 (56%)	4 (36%)	0.65
Perioperative HFV	4 (44%)	6 (55%)	1.0
Perioperative pneumothorax	0	2 (18%)	0.48
Age at CDH repair (d)	6.9 ± 2.0 (5–11)	5.9 ± 2.4 (2–11)	0.32
Patch CDH repair	9	11	
Survival to extubation	8 (89%)	9 (82%)	1.0
Age at extubation* (d)	37.9 ± 21.2 (21–87)	37.6 ± 15.7 (9–62)	0.63
Discharge on supplemental O_2	3 (43%)	4 (50%)	1.0
Overall survival†	7 (78%)	7 (64%)	0.64

Data are mean ± SD (range).

* All subjects survived to CDH repair. Deaths before extubation included one control subject from pulmonary hypertension (30 d), one TO subject from respiratory insufficiency (10 d), and one TO subject from sepsis (14 d).

† Two additional subjects died before discharge: one control subject from pulmonary hypertension and chronic lung disease (236 d) and one TO subject from pulmonary hypertension (71 d). One additional TO subject who was discharged on supplemental O_2 died from pulmonary hypertension after readmission (203 d).

All neonates were initially ventilated with 100% O_2, which was weaned as tolerated. Mechanical ventilation was managed to maintain an arterial carbon dioxide pressure (Pa_{CO_2}) in the range of 45–60 mm Hg (permissive hypercapnia) and right-hand (preductal) oxygen saturations ≥90%. Higher Pa_{CO_2} values and lower oxygen saturations were tolerated in the first hours of life. Right radial arterial catheters (pre–ductus arteriosus) were used for monitoring arterial oxygen pressure (Pa_{O_2}) values, if possible, particularly in the event of a large right-to-left shunt at the level of the ductus arteriosus. Pa_{O_2} alone was not used as a criterion for ventilator changes. Inhaled nitric oxide (iNO; INOmax; INO Therapeutics, Clinton, NJ) was administered for severe, prolonged arterial desaturation or failure to wean fraction of inspired oxygen (F_{IO_2}) over first several days of life. The starting dose of iNO was 20 ppm. A trial of high-frequency ventilation (HFV) was performed when we were unable to provide adequate ventilation or oxygenation at maximal conventional ventilator settings. HFV was used preferentially in cases of an air leak (pneumothorax in two TO cases). ECMO was also used as rescue therapy (one control subject). Newborns received additional surfactant doses when their clinical course and chest radiograph were consistent with hyaline membrane disease.

The diaphragmatic hernia was repaired when the respiratory status had stabilized with weaning of ventilator settings and F_{IO_2} (age 6.4 ± 2.2 d; Table 1). Infants remained pharmacologically paralyzed and sedated from birth until 24 h after CDH repair. After CDH repair, ventilator settings were weaned as tolerated, with liberal use of the pressure supported–assist control mode, and elective extubation of the trachea was attempted when low ventilator support was achieved. Once weaned from ventilatory support, infants remained on supplemental oxygen until able to maintain oxygen saturations (S_{PO_2}) >95% on room air. Infants who were unable to maintain S_{PO_2} >95% on room air were discharged on supplemental oxygen (Table 1).

Measurement of lung mechanics. Pulmonary function tests studies were performed at four time points: *1*) in the first 24 h after birth, *2*) within 24 h before CDH operative repair, *3*) within 24 h after CDH repair, and *4*) before elective tracheal extubation. Patients remained pharmacologically paralyzed for their clinical management through the first three measurements. The mean age at each study point did not differ between the control and TO groups (data not shown).

All studies were performed on mechanically ventilated infants using an in-line pneumotachometer connected to a neonatal respiratory function monitor (CO_2SMO Plus; Novametrix Medical Systems, Wallingford, CT). Ventilator PEEP was constant at all study points; it was adjusted on all subsequent studies to equal that of the initial study (2–3 cm H_2O). Infants who were on HFV were given manually assisted ventilation with bag and endotracheal tube for the purpose of the study at a PEEP of 2–3 cm H_2O (and equal to that of previous studies). The breath-to-breath measurements were downloaded directly to the CO_2SMO Plus system software. Measurements were subsequently exported to a spreadsheet (Excel; Microsoft, Redmond, WA), where 8–20 uniform, representative breaths (selected from tracings) were used for analysis. A summary measurement was obtained for the variables of interest from each study [respiratory system compliance (Crs), minute ventilation (V_E), and peak expiratory flow (PEF)] by calculating a mean value and then dividing by the patient's weight to normalize the measurements for body size (44).

Pulmonary gas exchange. Arterial pH and blood gas tensions (Pa_{CO_2}, Pa_{O_2}) measured for purposes of clinical management from birth through the perioperative period were gathered for analysis (4, 8, 12, 18, 24, 36, and 48 h of life; daily until CDH operative repair; 6, 12, 24, 36, and 48 h after surgery; and

on postoperative day 3). Blood source, ventilator settings, and pre- and post–ductus arteriosus S_{PO_2} differences were also collected. The single patient who required ECMO support was cannulated on the first day of life and decannulated preoperatively after 8 d of support. Arterial blood gas samples obtained while on ECMO were excluded as they did not reflect this patient's pulmonary gas exchange. Blood source was classified as "preductal" when it was obtained from the right upper extremity and "postductal" when it was obtained from the abdominal aorta or a lower extremity. Blood samples from the left radial artery (11% of total samples) were classified as preductal unless information from paired blood gas samples or simultaneous pre– and post–ductus arteriosus S_{PO_2} values were consistent with a postductal position (one of five patients). The majority of these samples were obtained in the postoperative period.

The following were calculated from the collected data:

$$\text{Oxygenation Index (OI)} = \text{Paw} \times \text{FiO}_2 \times 100/\text{PaO}_2 \quad (10)$$

$$\text{AaDO}_2 = [(\text{FiO}_2 \times (\text{P}_\text{B} - 47)) - \text{PaCO}_2/\text{RQ}] - \text{PaO}_2$$

Where P_{aw} is mean airway pressure, P_B = 760 mm Hg, 47 mm Hg is P_{H2O} at 37°C, and RQ = 1.

iNO response. The Pa_{O_2} just before beginning iNO and the subsequent values obtained at least 20 min after beginning therapy were recorded. Pre- and postductal S_{PO_2} were collected before and on iNO therapy. Because this was not a controlled study of iNO response, we chose stringent criteria to define an acute iNO responder: an increase in postductal S_{PO_2} of >5% *and* an increase in Pa_{O_2} of ≥50 mm Hg in arterial blood sampled from the same source. Previously, investigators have used an increase in Pa_{O_2} of >20 mm Hg as a definition of response to iNO in the CDH population (10).

Data analysis. Statistical analyses were performed with Stata 6.0 (Stata Corp., College Station, TX). Variables that were not normally distributed were analyzed with tests for nonparametric data. Statistical tests included *t* test, Mann-Whitney rank sum test, χ^2 or Fisher exact test, and linear regression. Repeated measures models were constructed using generalized estimating equations. Huber-White sandwich estimators of variance were used to allow robust standard errors estimations in the event of improper specification of the correlation structure between repeated measurements (45). The effect of study point was explored as a linear and quadratic function for all measures of lung mechanics. The natural log of gas exchange measures and P_{aw} changed linearly with time (age in hours); therefore, it was used as the outcome variable for construction of linear models. Results are presented as mean ± SD or percentage. P values presented in regression models are for individual predictor variable coefficients. All statistical tests were two-tailed, and results were considered significant at $p < 0.05$.

RESULTS

Lung mechanics. Crs (mL · cm H_2O^{-1} · kg^{-1}) was low in both groups. It was higher in the TO group at study 2 and study 4 (Table 2). There were no significant differences at any study point in comparing survivors and nonsurvivors or in classification of severity by LHR (≤0.90 *versus* >0.90; data not shown). Repeated measures models of Crs revealed that both treatment group and study point were significant predictors of Crs. Crs was higher in newborns who had undergone TO, it increased over time, and it increased at a faster rate in the TO group because of a significant interaction between treatment group and study point ($p = 0.007$; Fig. 1*A*). To evaluate bias as a result of death of three patients before study 4, we analyzed the model with exclusion of these three patients. The model remained significant with minor changes in the magnitude of the effects of our predictor variables.

There were no significant differences in V_E (mL · min^{-1} · kg^{-1}) at any study point by treatment group (Table 2), although V_E tended to be higher in TO infants. There were also no differences between infants who survived and those who died (data not shown). Analysis of V_E in relation to LHR revealed higher V_E in the low LHR group only at study 2 (257 ± 87 *versus* 171 ± 65 mL · min^{-1} · kg^{-1}; $p = 0.03$). After accounting for differences in the patients' Pa_{CO_2} during the study, this relationship was no longer significant ($p = 0.08$).

A trend toward a higher V_E in the TO group was revealed by repeated measures analysis ($\beta = 29$, $p = 0.05$). The study point was a significant predictor of V_E. In this model, V_E initially decreased (from study 1 to study 2) and then increased in the postoperative period (Fig. 1*B*). There was no interaction between treatment group and study point. After accounting for differences in the patients' Pa_{CO_2} during the study, the study point remained a significant predictor and the treatment effect was diminished ($\beta = 21$, $p = 0.17$); a lower Pa_{CO_2} was associated with higher V_E.

PEF was significantly higher in the TO group than in control subjects at all study points (Table 2). There were no differences

Table 2. *Lung mechanics at the four study points:* 1*) first 24 h after birth,* 2*) within 24 h before CDH operative repair,* 3*) within 24 h after CDH repair, and* 4*) before elective tracheal extubation*

	Crs (mL · cm H_2O^{-1} · kg^{-1})			V_E (mL · min^{-1} · kg^{-1})			PEF (L · min^{-1} · kg^{-1})		
	Control	TO	*p**	Control	TO	*p*†	Control	TO	*p**
Study 1	0.20 ± 0.06	0.22 ± 0.18	0.84	221 ± 55	259 ± 85	0.27	1.0 ± 0.1	1.8 ± 0.4	0.0006
Study 2	0.17 ± 0.04	0.28 ± 0.12	0.02	202 ± 80	212 ± 94	0.80	1.0 ± 0.2	1.6 ± 0.5	0.006
Study 3	0.24 ± 0.19	0.36 ± 0.25	0.19	182 ± 68	236 ± 69	0.10	1.0 ± 0.3	1.7 ± 0.5	0.03
Study 4	0.51 ± 0.16	0.93 ± 0.45	0.02	304 ± 78	314 ± 90	0.81	1.1 ± 0.3	1.8 ± 0.8	0.03

Data are mean ± SD.

* By Mann-Whitney rank sum test.

† By *t* test.

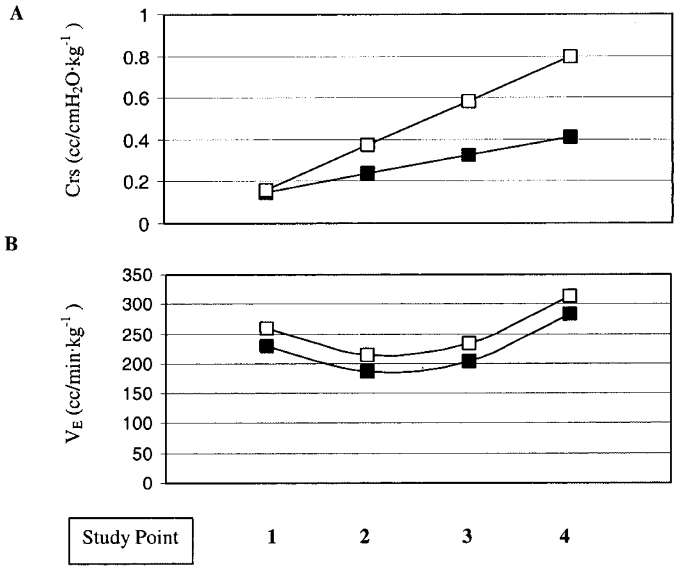

Figure 1. Repeated measures model of respiratory mechanics over the study period. Study points: *1*) first 24 h after birth, *2*) within 24 h before CDH operative repair, *3*) within 24 h after CDH repair, and *4*) before elective tracheal extubation. (*A*) Crs (mL · cm H_2O^{-1} · kg^{-1}) over the study period for each treatment group. There is a more rapid improvement in Crs over the study period in the TO group with a significant interaction between treatment group and study point. Regression equation: y = 0.06 − 0.11(treatment) + 0.09(study point) + 0.12(treatment × study point). (*B*) V_E (mL · min^{-1} · kg^{-1}) over the study period for each treatment group. Regression equation: y = 335 + 29(treatment) − 136(study point) + 31(study point)^2. □, Tracheal occlusion; ■, control.

in PEF between survivors and nonsurvivors or between LHR groups (data not shown). In repeated measures models, study point was not a significant predictor of PEF, but treatment group remained significant ($\beta = 0.65$, $p < 0.001$).

Pulmonary gas exchange. Representative values of OI and $AaDo_2$ at important clinical time points suggested that, as expected, OI and $AaDo_2$ decreased over time (Table 3). Univariate linear regression confirmed that the patient's age at the time of the sample had a significant correlation with these outcomes [decreasing OI ($p < 0.001$) and $AaDo_2$ ($p < 0.001$) with increasing age in hours]. Other significant associations with a lower OI were increased LHR ($p < 0.001$) and higher pH ($p < 0.001$). iNO therapy was associated with a higher OI ($p < 0.001$). For $AaDo_2$, increased LHR ($p < 0.001$) and a lower P_{aw} ($p < 0.001$) were associated with a lower $AaDo_2$. iNO therapy was associated with a higher $AaDo_2$ ($p < 0.001$).

In repeated measures models analysis of OI, treatment group was not a significant predictor variable ($p = 0.48$). OI significantly decreased with increasing age at the time of the sample (Fig. 2*A*). In contrast, TO was significantly associated with a lower $AaDo_2$ ($p = 0.006$). In addition, $AaDo_2$ decreased with advancing postnatal age (Fig. 2*B*). There was no significant interaction between treatment group and age. When LHR, blood sample site, and iNO therapy were added to the model, TO was associated with a lower $AaDo_2$ ($p = 0.009$) and treatment group still had no significant effect on OI ($p = 0.18$). LHR was an independent predictor of both OI ($p = 0.002$) and $AaDo_2$ ($p < 0.001$), with a decrease in these values with a higher LHR. A preductal blood gas source was significantly associated with a lower OI ($p = 0.009$) but not $AaDo_2$ ($p = 0.78$). iNO was not significantly associated with OI or $AaDo_2$ in these models.

In repeated measures models of other markers of gas exchange, P_{aw} significantly decreased with advancing postnatal age ($p = 0.01$), and there was a trend toward lower P_{aw} in the TO group ($p = 0.09$). $Paco_2$ was tightly controlled over the study period; there was no significant association of $Paco_2$ with postnatal age ($p = 0.87$) or treatment group ($p = 0.56$).

Seven (35%) of the patients had severe impairment of oxygenation (defined as OI >30) (10). This impairment persisted for >4 h in six of seven patients (three control subjects, three TO; $p = 1.0$).

iNO therapy. Nine patients (45%) were begun on iNO in the perioperative period (all before CDH repair; Table 1). The changes in Pao_2, OI, and postductal Spo_2 are shown in Figure 3. Three (33%) of the infants responded to iNO; all were control subjects. Although we chose more stringent criteria for an iNO response than previous investigators, in fact all patients whose Pao_2 increased >20 mm Hg also met our criteria by increasing their Pao_2 ≥50 mm Hg and their postductal Spo_2 >5%. The single patient who required ECMO support was a nonresponder.

DISCUSSION

We have demonstrated a modest improvement in newborn lung function after fetal TO for severe left CDH. These improvements consisted of increased Crs, increased PEF, and increased oxygenation, as measured by $AaDo_2$. This is the first report of these physiologic measurements after fetal TO in humans. It is also the first study that compares these measurements in infants who had fetal TO with infants who had a

Table 3. *OI and $AaDo_2$ at important clinical time points in the control and TO groups*

	4 h	24 h*	48 h*	Day 3*	Preoperative	6 h postoperative	Day 3 postoperative
Postnatal age (h)	4 ± 1	24 ± 1	49 ± 1	60 ± 6	140 ± 51	156 ± 53	211 ± 52
OI							
Control	32 ± 29	13 ± 11	16 ± 10	9 ± 4	7 ± 2	6 ± 3	7 ± 4
TO	18 ± 13	16 ± 16	10 ± 10	9 ± 9	7 ± 5	6 ± 6	6 ± 5
$AaDo_2$							
Control	547 ± 127	468 ± 139	496 ± 128	436 ± 116	310 ± 94	305 ± 81	250 ± 110
TO	418 ± 180	322 ± 175	274 ± 171	250 ± 187	199 ± 173	183 ± 165	166 ± 166

Data are mean ± SD.

* Values exclude the control subject who was on ECMO support.

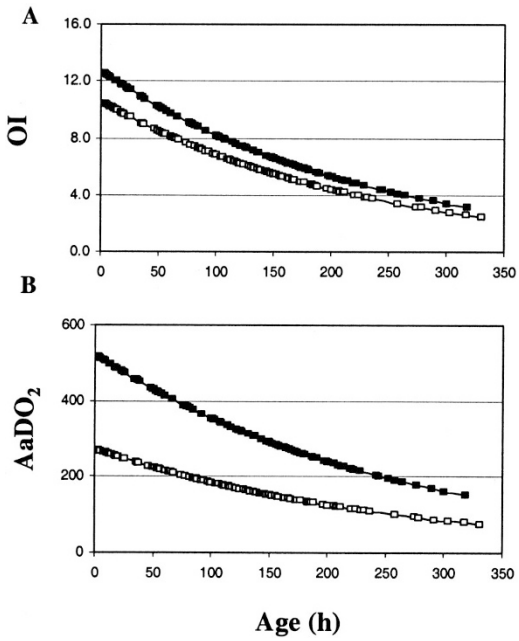

Figure 2. Repeated measures models of measures of oxygenation *vs* postnatal age by treatment group. (A) OI does not differ by treatment group. Regression equation for ln(OI): y = 2.542 − 0.185(treatment) − 0.004(age). Values plotted are e^y. (B) $AaDo_2$ is lower in the TO group. Regression equation for ln($AaDo_2$): y = 6.257 − 0.651(treatment) − 0.004(age). Values plotted are e^y. □, Tracheal occlusion; ■, control.

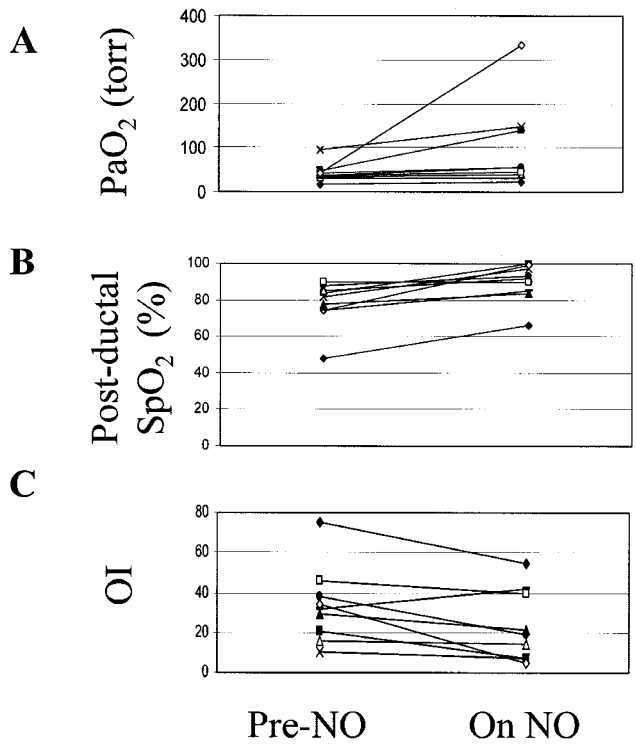

Figure 3. Response of Pao_2, OI, and postductal Spo_2 to initiation of therapy in the nine subjects who were treated with iNO.

similar degree of severity of CDH and received conventional postnatal care. Furthermore, these are the first longitudinal, extended measurements of lung function in any population after fetal TO. The findings are intriguing, given the biologic and physiologic effects of TO noted in animal models of CDH.

These elegant animal experiments have clearly documented intrauterine lung growth and morphologic maturation, with increased area for gas exchange (28,30,32,35,46) and variable effects on gas exchange with short-term assisted ventilation postnatally (28,36). In our study, the compliance measures were extremely low (47), which other investigators have previously documented in CDH (21,23,24,48). Therefore, even a small impact on lung compliance could be clinically significant in this population. We did not perform lung volume measurements by gas dilution or lung morphologic examinations. However, the modest increases in Crs in this study could be secondary to increased fetal lung growth in the TO group (44). Although V_E had a wide range, the values were in the normal range (47) and were highly associated with $Paco_2$ (higher V_E associated with lower $Paco_2$), which was tightly controlled throughout the perioperative period.

Nakayama *et al.* (49) used the deflation flow volume curve technique and passive expiratory flow volume curves to assess airway obstruction and resistance in paralyzed, intubated newborns with CDH in the first month of life after repair and stabilization. They found that this population did have significant airway obstruction that was partially reversible through bronchodilator therapy. An increased propensity to bronchial constriction was recently demonstrated in the nitrofen-induced CDH model in rats, which was associated with increased cyclooxygenase in the airway smooth muscle cells (50). Therefore, the higher PEF measures after TO may demonstrate some effect of fetal intervention on airway remodeling. We corrected these measurements to the patients' weight to standardize the measurements for size. However, previous investigators have demonstrated that airway conductance does not have a linear relationship to size in healthy newborns, with the airways relatively more developed in small infants (44). Therefore, the greater PEF that we observed in the TO group may be secondary to prematurity rather than an effect of TO itself. Conversely, the endotracheal tube internal diameter may contribute significantly to these measurements. This effect would favor lower flow in the smaller TO group with narrower, higher resistance tubes (51).

Oxygenation is affected by parenchymal lung disease (atelectasis, edema, pneumothorax), alveolar surface area, and the pulmonary vasculature. The resistance of the pulmonary vasculature determines pulmonary blood flow, and in CDH, the resistance is determined by the size of the pulmonary vascular bed as well as its degree of vasodilation (25). Thickening of the muscular layer of the pulmonary arteries occurs as early as 21 wk GA in fetuses with CDH and pulmonary hypoplasia (2). TO in experimental animals with CDH accelerates lung growth (30,35) and tends to normalize the pulmonary vascular morphology (27,29,52) and function (31,36). In the current study, TO fetuses had better oxygenation (as measured by $AaDo_2$). This may have been due to increased lung size, more normal pulmonary vasculature, or contributions of both effects.

Other factors may explain differences in oxygenation. Surfactant administration differed between groups, with a higher proportion of the TO group receiving prophylactic surfactant (Table 1). Surfactant administration in the ovine CDH model improves gas exchange; this has been attributed to improve-

ments in lung mechanics (53) and pulmonary vascular resistance (54). However, it is unlikely that surfactant therapy accounts for the differences seen between the two groups because we would expect to see more of an effect early in the course. Another factor is related to the developmental changes in smooth muscle in the pulmonary vasculature. In preterm infants without CDH, the pulmonary vascular smooth muscle is less developed than that of term infants. The changes in muscularization through advancing gestation in CDH are unknown in humans, but the ovide model supports progressive pulmonary hypoplasia with advancing gestation (55). Thus, the effect of prematurity in CDH may result in increased oxygenation. Ventilator strategies will also affect oxygenation. The ventilator management was consistent between groups, but there was a tendency to use lower P_{aw} in the smaller TO group (which may account for the failure to see a difference in oxygenation through evaluation of OI). Our studies do not allow us to determine the mechanism by which TO increased oxygenation.

The significant association that we saw of decreased oxygenation with lower LHR [which may be a proxy for intrauterine lung volume (56)] is an important observation. This could be related to smaller lung volume with lower LHR or a more abnormal pulmonary vasculature with lower LHR, as more pronounced vascular abnormalities are seen in more severe lung hypoplasia (4).

The ability to respond to iNO therapy (seen in three subjects from the control group) points to a reactive pulmonary vascular bed as a cause of hypoxemia in a subset of the subjects. Newborns with CDH have variable responses to vasodilator therapy (8,10). The proportion of NO responders in our study cohort was similar to what has previously been seen (16%) in the controlled setting in the CDH population (10).

CDH is a heterogeneous condition with varying degrees of anatomic severity and lung hypoplasia (23,38). Although we may have been able to decrease this heterogeneity through our strict antenatal inclusion criteria, it is likely to have been compounded by other sources of variability between and within the treatment groups. All subjects in the TO group were preterm, and all subjects in the control group were term, with no overlap between the GAs of these groups. We attempted to account for some of this difference through adjustment of lung mechanics measurements for body weight. However, we could not take into account other differences between the subjects, and the strong association of prematurity and fetal intervention confounds our ability to evaluate effects of GA on lung function. The more compliant chest wall of the preterm newborn would result in a relative increase in compliance in the TO group. Because subjects were pharmacologically paralyzed through the postoperative measurements (study 3), this effect should be less pronounced. An additional confounder was the wide range of duration of TO (16–64 d). This was also significantly correlated with the degree of prematurity. There are likely differing biologic effects of TO in humans, dependent on its duration (lung growth and differentiation, surfactant production, vascular growth and remodeling), as there are in animal models (30,46,52). These effects may differ longitudinally, as the intrauterine and postnatal development and maturation of the lung in CDH are likely related to the degree of prematurity and duration of TO.

In this study, we have not been able to draw conclusions regarding the mechanisms of effects of fetal TO from either an anatomic or a biochemical perspective. Despite the limitations of this study population, our findings describe possible benefits of fetal TO in this population, with small, measurable differences in lung function. However, in view of the absence of any significant effect on survival, these modest improvements in pulmonary function in infants after TO are of questionable clinical significance.

Acknowledgments. We are grateful to Paul Gardner and the UCSF Neonatal Clinical Physiology Laboratory for technical support and to the nurses from the UCSF Fetal Treatment Center and the Neonatal Satellite of the Pediatric Clinical Research Center for assistance with data collection.

REFERENCES

1. Skari H, Bjornland K, Haugen G, Egeland T, Emblem R 2000 Congenital diaphragmatic hernia: a meta-analysis of mortality factors. J Pediatr Surg 35:1187–1197
2. Barghorn A, Koslowski M, Kromminga R, Hufnagl P, Tennstedt C, Vogel M 1998 Alpha-smooth muscle actin distribution in the pulmonary vasculature comparing hypoplastic and normal fetal lungs. Pediatr Pathol Lab Med 18:15–22
3. Kitigawa M, Hislop A, Boyden EA, Reid L 1971 Lung hypoplasia in congenital diaphragmatic hernia. A quantitative study of airway, artery, and alveolar development. Br J Surg 58:342–346
4. Naeye R, Shochat SJ, Whitman V, Maisels MJ 1976 Unsuspected pulmonary vascular abnormalities associated with diaphragmatic hernia. Pediatrics 58:902–906
5. Thibeault D, Haney B 1998 Lung volume, pulmonary vasculature, and factors affecting survival in congenital diaphragmatic hernia. Pediatrics 101:289–295
6. Redmond C, Heaton J, Calix J, Graves E, Farr G, Falterman K, Arensman R 1987 A correlation of pulmonary hypoplasia, mean airway pressure, and survival in congenital diaphragmatic hernia treated with extracorporeal membrane oxygenation. J Pediatr Surg 22:1143–1149
7. Allan DW, Greer JJ 1997 Pathogenesis of nitrofen-induced congenital diaphragmatic hernia in fetal rats. J Appl Physiol 83:338–347
8. Bos AP, Tibboel D, Koot VC, Hazebroek FW, Molenaar JC 1993 Persistent pulmonary hypertension in high-risk congenital diaphragmatic hernia patients: incidence and vasodilator therapy. J Pediatr Surg 28:1463–1465
9. Karamanoukian HL, Glick PL, Zayek M, Steinhorn RH, Zwass MS, Fineman JR, Morin FC 3rd 1994 Inhaled nitric oxide in congenital hypoplasia of the lungs due to diaphragmatic hernia or oligohydramnios. Pediatrics 94:715–718
10. The Neonatal Inhaled Nitric Oxide Study Group 1997 Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. Pediatrics 99:838–845
11. Kinsella JP, Truog WE, Walsh WF, Goldberg RN, Bancalari E, Mayock DE, Redding GJ, deLemos RA, Sardesai S, McCurnin DC, Moreland SG, Cutter GR, Abman SH 1997 Randomized, multicenter trial of inhaled nitric oxide and high-frequency oscillatory ventilation in severe, persistent pulmonary hypertension of the newborn. J Pediatr 131:55–62
12. Wilson JM, Lund DP, Lillehei CW, Vacanti JP 1997 Congenital diaphragmatic hernia-a tale of two cities: the Boston experience. J Pediatr Surg 32:401–405
13. Bohn D, Tamura M, Perrin D, Barker G, Rabinovitch M 1987 Ventilatory predictors of pulmonary hypoplasia in congenital diaphragmatic hernia, confirmed by morphologic assessment. J Pediatr 111:423–431
14. Azarow K, Messineo A, Pearl R, Filler R, Barker G, Bohn D 1997 Congenital diaphragmatic hernia—a tale of two cities: the Toronto experience. J Pediatr Surg 32:395–400
15. Boloker J, Bateman D, Wung JT, Stolar CJ 2002 Congenital diaphragmatic hernia in 120 infants treated consecutively with permissive hypercapnea/spontaneous respiration/elective repair. J Pediatr Surg 37:357–366
16. Bohn D 2002 Congenital diaphragmatic hernia. Am J Respir Crit Care Med 166:911–915
17. Downward CD, Jaksic T, Garza JJ, Dzakovic A, Nemes L, Jennings RW, Wilson JM 2003 Analysis of an improved survival rate for congenital diaphragmatic hernia. J Pediatr Surg 38:729–732
18. Stege G, Fenton A, Jaffray B 2003 Nihilism in the 1990s: the true mortality of congenital diaphragmatic hernia. Pediatrics 112:532–535
19. The Congenital Diaphragmatic Hernia Study Group 2001 Estimating disease severity of congenital diaphragmatic hernia in the first 5 minutes of life. J Pediatr Surg 36:141–145
20. Sakai H, Tamura M, Hosokawa Y, Bryan AC, Barker GA, Bohn DJ 1987 Effect of surgical repair on respiratory mechanics in congenital diaphragmatic hernia. J Pediatr 111:432–438

21. Tracy TF Jr, Bailey PV, Sadiq F, Noguchi A, Silen ML, Weber TR 1994 Predictive capabilities of preoperative and postoperative pulmonary function tests in delayed repair of congenital diaphragmatic hernia. J Pediatr Surg 29:265–270
22. Nakayama DK, Motoyama EK, Tagge EM 1991 Effect of preoperative stabilization on respiratory system compliance and outcome in newborn infants with congenital diaphragmatic hernia. J Pediatr 118:793–799
23. Antunes MJ, Greenspan JS, Cullen JA, Holt WJ, Baumgart S, Spitzer AR 1995 Prognosis with preoperative pulmonary function and lung volume assessment in infants with congenital diaphragmatic hernia. Pediatrics 96:1117–1122
24. Kavvadia V, Greenough A, Laubscher B, Dimitriou G, Davenport M, Nicolaides KH 1997 Perioperative assessment of respiratory compliance and lung volume in infants with congenital diaphragmatic hernia: prediction of outcome. J Pediatr Surg 32:1665–1669
25. Vacanti JP, Crone RK, Murphy JD, Smith SD, Black PR, Reid L, Hendren WH 1984 The pulmonary hemodynamic response to perioperative anesthesia in the treatment of high-risk infants with congenital diaphragmatic hernia. J Pediatr Surg 19:672–679
26. Haugen SE, Linker D, Eik-Nes S, Kufaas T, Vik T, Eggen BM, Brubakk AM 1991 Congenital diaphragmatic hernia: determination of the optimal time for operation by echocardiographic monitoring of the pulmonary arterial pressure. J Pediatr Surg 26:560–562
27. Kanai M, Kitano Y, von Allmen D, Davies P, Adzick NS, Flake AW 2001 Fetal tracheal occlusion in the rat model of nitrofen-induced congenital diaphragmatic hernia: tracheal occlusion reverses the arterial structural abnormality. J Pediatr Surg 36:839–845
28. Hedrick MH, Estes JM, Sullivan KM, Bealer JF, Kitterman JA, Flake AW, Adzick NS, Harrison MR 1994 Plug the lung until it grows (PLUG): a new method to treat congenital diaphragmatic hernia in utero. J Pediatr Surg 29:612–617
29. Luks FI, Wild YK, Piasecki GJ, De Paepe ME 2000 Short-term tracheal occlusion corrects pulmonary vascular anomalies in the fetal lamb with diaphragmatic hernia. Surgery 128:266–272
30. Lipsett J, Cool JC, Runciman SI, Ford WD, Kennedy JD, Martin AJ 1998 Effect of antenatal tracheal occlusion on lung development in the sheep model of congenital diaphragmatic hernia: a morphometric analysis of pulmonary structure and maturity. Pediatr Pulmonol 25:257–269
31. Sylvester KG, Rasanen J, Kitano Y, Flake AW, Crombleholme TM, Adzick NS 1998 Tracheal occlusion reverses the high impedance to flow in the fetal pulmonary circulation and normalizes its physiological response to oxygen at full term. J Pediatr Surg 33:1071–1074
32. Wu J, Ge X, Verbeken EK, Garatacos E, Yesildaglar N, Deprest JA 2002 Pulmonary effects of in utero tracheal occlusion are dependent on gestational age in a rabbit model of diaphragmatic hernia. J Pediatr Surg 37:11–17
33. Joe P, Wallen LD, Chapin CJ, Lee CH, Allen L, Han VK, Dobbs LG, Hawgood S, Kitterman JA 1997 Effects of mechanical factors on growth and maturation of the lung in fetal sheep. Am J Physiol 272:L95–L105
34. Piedboeuf B, Laberge JM, Ghitulescu G, Gamache M, Petrov P, Belanger S, Chen MF, Hashim E, Possmayer F 1997 Deleterious effect of tracheal obstruction on type II pneumocytes in fetal sheep. Pediatr Res 41:473–479
35. Benachi A, Chailley-Heu B, Delezoide AL, Dommergues M, Brunelle F, Dumez Y, Bourbon JR 1998 Lung growth and maturation after tracheal occlusion in diaphragmatic hernia. Am J Respir Crit Care Med 157:921–927
36. Wild YK, Piasecki GJ, De Paepe ME, Luks FI 2000 Short-term tracheal occlusion in fetal lambs with diaphragmatic hernia improves lung function, even in the absence of lung growth. J Pediatr Surg 35:775–779
37. Davey MG, Hedrick HL, Bouchard S, Mendoza JM, Schwarz U, Adzick NS, Flake AW 2003 Temporary tracheal occlusion in fetal sheep with lung hypoplasia does not improve postnatal lung function. J Appl Physiol 94:1054–1062
38. Harrison MR, Keller RL, Hawgood SB, Kitterman JA, Sandberg PL, Farmer DL, Lee H, Filly RA, Farrell JA, Albanese CT 2003 A randomized trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. N Engl J Med 349:1916–1924
39. Lipshutz GS, Albanese CT, Feldstein VA, Jennings RW, Housley HT, Beech R, Farrell JA, Harrison MR 1997 Prospective analysis of lung-to-head ratio predicts survival for patients with prenatally diagnosed congenital diaphragmatic hernia. J Pediatr Surg 32:1634–1636
40. VanderWall K, Skarsgard E, Filly RA, Eckert J, Harrison MR 1997 Fetendo-clip: a fetal endoscopic tracheal clip procedure in a human fetus. J Pediatr Surg 32:970–972
41. Harrison MR, Albanese CT, Hawgood SB, Farmer DL, Farrell JA, Sandberg PL, Filly RA 2001 Fetoscopic temporary tracheal occlusion by means of detachable balloon for congenital diaphragmatic hernia. Am J Obstet Gynecol 185:730–733
42. Boland RB, Nardo L, Hooper SB 1997 Cortisol pretreatment enhances the lung growth response to tracheal obstruction in fetal sheep. Am J Physiol 273:L1126–L1131
43. Mychaliska GB, Bealer JF, Graf JL, Rosen MA, Adzick NS, Harrison MR 1997 Operating on placental support: the ex utero intrapartum treatment procedure. J Pediatr Surg 32:227–230
44. Gerhardt T, Hehre D, Feller R, Reifenberg L, Bancalari E 1987 Pulmonary mechanics in normal infants and young children during first 5 years of life. Pediatr Pulmonol 3:309–316
45. Dupont W 2002 Statistical Modeling for Biomedical Researchers. Cambridge University Press, Cambridge, UK
46. Papadakis K, De Paepe ME, Tackett LD, Piasecki GJ, Luks FI 1998 Temporary tracheal occlusion causes catch-up lung maturation in a fetal model of diaphragmatic hernia. J Pediatr Surg 33:1030–1037
47. Chu J, Clements J, Cotton E, Klaus M, Sweet A, Tooley W1967 Neonatal pulmonary ischemia. Part I: clinical and physiological studies. Pediatrics 40(suppl 4):709–782
48. Dimitriou G, Greenough A, Chan V, Gamsu H, Howard ER, Nicolaides KH 1995 Prognostic indicators in congenital diaphragmatic hernia. J Pediatr Surg 30:1694–1697
49. Nakayama DK, Motoyama EK, Mutich RL, Koumbourlis AC 1991 Pulmonary function in newborns after repair of congenital diaphragmatic hernia. Pediatr Pulmonol 11:49–55
50. Belik J, Davidge ST, Zhang W, Pan J, Greer JJ 2003 Airway smooth muscle changes in the nitrofen-induced congenital diaphragmatic hernia rat model. Pediatr Res 53:737–743
51. Wright PE, Marini JJ, Bernard GR 1989 In vitro versus in vivo comparison of endotracheal tube airflow resistance. Am Rev Respir Dis 140:10–16
52. Bratu I, Flageole H, Laberge JM, Chen MF, Piedboeuf B 2001 Pulmonary structural maturation and pulmonary artery remodeling after reversible fetal ovine tracheal occlusion in diaphragmatic hernia. J Pediatr Surg 36:739–744
53. Wilcox DT, Glick PL, Karamanoukian H, Rossman J, Morin FC 3rd, Holm BA 1994 Pathophysiology of congenital diaphragmatic hernia V. E effect of exogenous surfactant therapy on gas exchange and lung mechanics in the lamb congenital diaphragmatic hernia model. J Pediatr 124:289–293
54. O'Toole SJ, Karamanoukian HL, Morin FC 3rd, Holm BA, Egan EA, Azizkhan RG, Glick PL 1996 Surfactant decreases pulmonary vascular resistance and increases pulmonary blood flow in the fetal lamb model of congenital diaphragmatic hernia. J Pediatr Surg 31:507–511
55. Lipsett J, Cool JC, Runciman SI, Ford WD, Kennedy JD, Martin AJ, Parsons DW 2000 Morphometric analysis of preterm fetal pulmonary development in the sheep model of congenital diaphragmatic hernia. Pediatr Dev Pathol 3:17–28
56. Paek BW, Coakley FV, Lu Y, Filly RA, Lopoo JB, Qayyum A, Harrison MR, Albanese CT 2001 Congenital diaphragmatic hernia: prenatal evaluation with MR lung volumetry-preliminary experience. Radiology 220:63–67