

*Original Article***Analysis of lamb lung development with tracheal ligation in left posterolateral diaphragmatic hernia in cases and controls**

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Abstract

BACKGROUND: When we perform surgery in utero, lungs have an appropriate time to decrease magnitude of hypoplasia, with surgery in uterus.

METHODS: Six time-dated single-fetus ewes were selected to induce diaphragmatic hernia. Fetal lambs were divided proportionally into 2 groups, namely group 1, diaphragmatic hernia and tracheal ligation (TL group), and group 2, diaphragmatic hernia only (NL group). Morphologic assessments (weight, volume, bronchiolar branching) and histological tests (lung maturation phase) were performed.

RESULTS: Lung weight in relation to lamb weight in the tracheal ligation group differed significantly from the control group ($P < 0.006$ in the right lung and $P < 0.005$ in the left lung). Other parameters were markedly different between the two groups. Lung volumes in relation to lamb weight were 29.88 ± 12.54 in the tracheal ligated group [TL] and 6.44 ± 1.84 in the non-ligated [NL] group in the right lung and 21.52 ± 7.58 in the TL group and 3.86 ± 1.90 in the NL group in the left lung. All parameters were in favor of tracheal ligation. Lung maturation phase arrested in the canalicular phase in the NL group and continued to the saccular phase in the TL group.

CONCLUSIONS: Tracheal ligation resulted in more mature lungs in the TL group.

KEY WORDS: Congenital diaphragmatic hernia, tracheal ligation, sheep, lamb, fetal surgery.

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Congenital diaphragmatic hernia (CDH) is one of the most challenging neonatal diagnoses faced by pediatric surgeons. Despite increased diagnostic capabilities and tremendous advances in the care and management of critically ill neonates with respiratory disease, CDH still has an overall mortality of 30 to 60% in most centers. The high mortality of CDH is directly related to the severity of lung hypoplasia induced by bowel herniation during the critical stages of fetal lung development.

Perhaps no other disease encountered by pediatric surgeons has as many therapeutic and management options as do CDH. Regardless, the optimal management strategy for neonates with congenital diaphragmatic hernia remains unknown, and the treatment of CDH continues to evolve as our understanding of the disease increases¹. We attempted to examine our capability to perform surgery before the beginning of the complications of CDH on lung in the fetal period.

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Methods

This is an empirical prospective study. We selected six time-dated ewes at 80 days post conception (full term ~148) as determined by radiology and sonography by a specialist in animal radiology². All ewes were 2-3 years old and all work was done in the veterinary hospital of Shahrekord Azad University in winter 2005. The ewes were kept in fasting state for 48 hours before surgery. We used indomethacin suppository as tocolytic; then the right aspect of thorax and abdomen were shaved and surgery was conducted.

Surgical techniques

The ewe was placed on operation table in left lateral decubitus position. Intravenous cannula was placed in left external jugular vein and the animal was premedicated with intravenous thiopental (1 g). The ewe was then intubated by orotracheal tube (size, ID = 9 mm) and anesthesia was continued by halothane (2-4%) and nitrous oxide in inspired air. Before surgery, we infused cefazolin (1 g) intravenously as prophylactic antibiotic. Then, caudoventral incision on the left aspect of the abdomen was made and laparotomy was done by electrocautery. The womb was found and the fetus position was determined by palpation³. The womb was incised 5-6 cm on the upper left aspect of the fetus's thorax and amniotic sac was sutured to the myometrium to prevent retraction of sac.

While under general maternal anesthesia, the left aspect of thorax was located and a 3-4 cm incision was made on the lower third of thorax on lower ribs and the lung was retracted and a circular incision, 2 cm in diameter was made in the diaphragm. Stomach and small bowel were pulled into the thorax to create a diaphragmatic hernia²⁻⁵. The thoracotomy incision was closed by 2-0 chromic catgut suture in continuous manner. Skin was closed by 4-0 nylon suture³.

In the control group, surgery on the fetus was terminated and in the case group, lower neck was incised transversely and trachea was located by palpation and ligated with heavy silk suture (USP 1) and skin was closed with 4-0 nylon suture. The amniotic sac and the myo-

metrium were then repaired in 2 layers with 2-0 chromic catgut and approximately 200-300 ml warm lactated Ringer's solution was infused into the amniotic sac to replace amniotic fluid and then one million units of penicillin G was infused into the amniotic fluid. Myometrium was checked for amniotic fluid leakage; if present, the leak would be sealed by figure of eight 2-0 chromic catgut suture.

The abdominal wall was closed in layers by chromic catgut (2 USP) and the skin was closed by 2-0 nylon sutures. Wound was sprayed by oxytetracycline antibiotic as dressing.

Inspiratory anesthetic gases were discontinued and the ewe was extubated.

After surgery, intravenous access was preserved for three days and cefazolin (1 g) was administered every eight hours intravenously for the first day and indomethacin suppository was used until lambing every eight hours. Serum DW 5% in 1/3 normal saline was infused to the ewes until they could eat appropriately; then intravenous access was extracted³⁻⁸.

One day after surgery, all of the ewes underwent sonography for evaluation of lamb health³. Then sonography was repeated weekly to monitor lamb health.

After lambing

After lambing, the lambs in the case group died because their trachea had been ligated and they could not breathe. The lambs in the control group were terminated after birth. Fetuses were fixed with 10% formalin and then autopsied. First, a sample of the lung, approximately 1-2 milliliters in volume, was separated for paraffin embedding block preparation from lingula and the right middle lobe^{3,5,9-11}.

Statistical Analysis

Statistical evaluation of the results was done by Student's t-test.

Results

Morphological analysis

We weighed the lambs in both groups and compared mean values by Student's t-test but we found no significant difference between the

two groups. Body weight in the tracheal ligated group was lower than in the non-ligated group (2.08 ± 1.71 kg in the ligated group versus 3.34 ± 1.39 in the non-ligated group). We also weighed other organs separately (like the lungs, heart, kidneys and adrenals), but none differed significantly between the two groups. Organs like the lungs and heart weighed more in the tracheal ligated group but statistics showed no significant difference between the two groups.

We compared the relationships between lung weight and volume to body weight (as shown in table 1) and compared these relationships with each other within each group. Re-

sults are shown in table 2. We found a significant difference between the two groups. The relationship between the weight of each lung to body weight was significantly stronger in the tracheal ligated group (P value <0.006 for the right lung and P value <0.005 for the left lung). The relationship between lung volumes and body weight was also stronger in the tracheal ligated group but was not significantly different from the other group as shown by Student's t-test. Bronchiolar branching was not significantly different between the two groups; neither was there any difference between the right and left lungs in each lamb.

Table 1. Relationship between the weights of different organs to body weight

	Right lung weight/body weight	Left lung weight/body weight	Heart weight/body weight	Right lung volume/body weight	Left lung volume/body weight
A(T.L)	28.72	16.54	6.36	25.64	15.38
B(N.L)	36.40	25.20	7.20	44.00	30.00
C(N.L)	22.00	17.51	17.03	20.00	19.19
D(T.L)	7.71	5.54	5.88	8.33	5.83
E(N.L)	6.34	3.69	5.56	6.34	3.73
F(T.L)	4.40	2.08	5.74	4.65	2.02

Table 2. Comparison of the relationship between the weights of different organs to body weight in the two groups

	Right lung weight/body weight *	Left lung weight/body weight **	Heart weight/body weight	Right lung volume/body weight	Left lung volume/body weight
TL group	29.03 ± 7.20	19.75 ± 4.74	10.19 ± 5.93	29.88 ± 12.54	21.52 ± 7.58
NL group	6.15 ± 1.66	3.77 ± 1.73	5.72 ± 0.15	6.44 ± 1.84	3.86 ± 1.90

Histological analysis

Histological analysis was performed with light microscopy. In the non-ligated group, maturation

arrested in the canalicular phase and in the ligated group lung maturation stage was in saccular phase in all samples (table 3).

Table 3. Comparison between maturation stages in the two groups

	Pseudoglandular phase	Canalicular phase	Saccular phase
TL group	0	0	3
NL group	0	3	0

Analysis of Ewes

All of the mothers were terminated at the end of the study and the abdomen and uterus were dissected. Minimal adhesion formation oc-

curred in the two cases. One of them had synechia of uterine endothelium. Repaired uterine wall healed completely and none of them had disruption of the uterine wall.

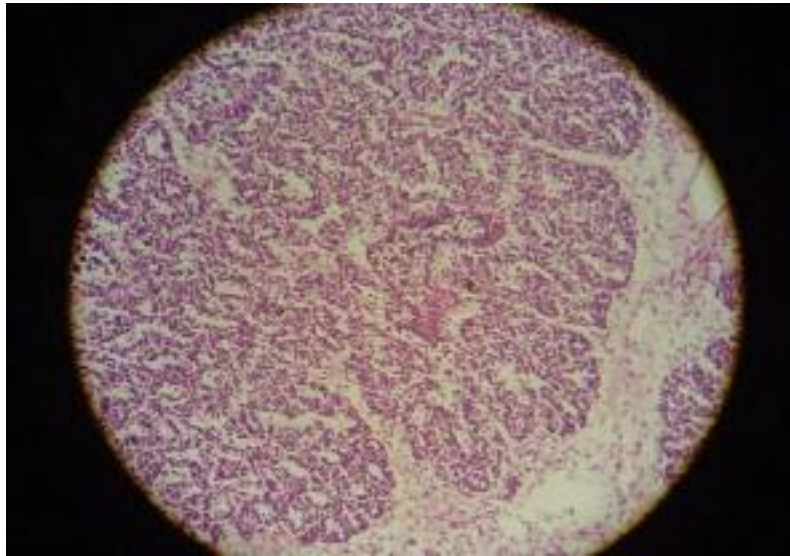


Figure 1. Immature lung view in the control group.



Figure 2. Mature lung view in the case group

Discussion

Flow of fluid from the lungs to amniotic fluid was obstructed with ligation of the trachea³. In the uterus, the lungs are fluid-producing organs until a few days before term. The rate of fluid production in fetal sheep has been estimated to be 10-14 ml/h or 4.5 ml/kg/h^{3,12}. Fetuses born with tracheal atresia were found to have lungs that were enlarged and hyperplastic at macroscopic and microscopic levels, respectively. Experimental fetal tracheal ligation

has been shown to cause augmented lung growth before birth and reduce the hernia in the CDH animal model. The effect is believed to be secondary to increased lung fluid retention and elevated tracheal fluid pressure, although the effects on pressure have never been adequately studied³.

In our study, lung growth increased by volume and weight with the obstruction of the flow of fluid by tracheal ligation. In another study, tracheal ligation increased pressure dif-

ferences between trachea and amniotic fluid to 4-6 mmHg^{13,14}. Recent articles advise mothers of fetuses with hypoplastic lungs to exercise multiple straining and the Valsalva maneuver to increase amniotic fluid pressure to stimulate fetal lung growth¹⁵. In histologic analysis, we observed an increase in alveolar count and more mature alveolar appearance at the microscopic level (figures 1 and 2). Heart and other organs are not influenced by tracheal ligation. In our study heart weight and anatomy were normal and unchanged by surgery¹⁶. Bronchiolar branching had not been changed by tracheal ligation¹⁷ probably because most of the branching had occurred in pseudoglandular and canalicular phases of lung maturation before our intervention. Pseudoglandular and canalicular phases occurred before midterm of pregnancy and our intervention and the sacular phase occurred from midterm to birth. The alveolar phase occurred after birth¹. Histological analysis was used to compare lung architecture. Lung growth in response to tracheal obstruction results in an increased number of lung units and increases in the size of preexisting units (figures 1 and 2).

The air space fraction was significantly higher in the case group and alveolar wall was thinner; characteristic found in more mature lungs^{17,18}. Histological analysis in the non-ligated group showed that lung maturation had arrested in the canalicular phase. The lungs had reached this degree of maturation by midterm of fetal life before our intervention. However, in the non-ligated group, maturation stopped and in the tracheal ligated group continued as in a normal lamb. Probably this was the most important event in our study that shows the efficacy of tracheal ligation. It is evident from this study and others that prevention of fluid egresses from the potential airspaces in the developing fetus lungs results in continued lung development. One proposed mechanism of action is the modulation of local growth factors via airspace pressure receptors.

An increase in lung fluid pressure may

augment a local growth cascade. The effect on growth has been shown to confine itself strictly to areas of lung fluid retention in this mechanism. Systemically circulating growth factors would probably be ineffective without tracheal obstruction^{3,6}. Whereas pressure tends to cause necrosis of tissue (as frequently seen with abscesses and tumor growth) tension can increase the mitotic activity in various tissues. Several investigators have examined epithelial cell division in response to tension. Increased mechanical stress applied to cultured fibroblasts was shown to increase the mitotic activity by speeding up the cell cycle^{2,3}. Others observed the effect of applied tension *in vivo* on mouse skin and confirmed increased mitotic activity in response to this intervention. Liu et al studied the effect of mechanical stretch on proliferation of fetal rat lung cells. They concluded that mechanical forces act directly to stimulate fetal rat lung cell growth and this effect is not mediated by prostaglandins or leukotrienes. The concept of tension affecting tissue growth has been applied to clinical practice for several years with use of skin expanders. With fetal surgery becoming a reality in the clinical setting, methods of minimizing the surgical complications are being investigated. One of the gravest complications is preterm labor, which is often difficult to control medically. The uterine instability is believed to be secondary to the hysterotomy wound³. In our study, we did not have any cases of preterm labor, threatened abortion or amniotic fluid leakage; this shows fetal surgery to be a safe practice with low complication rates and may serve as a prelude to fetal surgery even in humans in future studies. It seems logical that reducing the size of the wound may decrease dreaded complications. Modern technology has allowed minimally invasive surgery with laparoscopic equipment, and this has recently been used in the experimental setting in fetal surgery. Our tracheal occlusion technique could easily be modified to incorporate the use of these techniques³.

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