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## HEART HYPOPLASIA IN AN ANIMAL MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA

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TANNURI U - Heart hypoplasia in an animal model of congenital diaphragmatic hernia. *Rev. Hosp. Clín. Fac. Med. S. Paulo* 56(6):173-178, 2001.

**Purpose:** In previous papers, we described a new experimental model of congenital diaphragmatic hernia in rabbits, and we also reported noninvasive therapeutic strategies for prevention of the functional and structural immaturity of the lungs associated with this defect. In addition to lung hypoplasia, pulmonary hypertension, biochemical, and structural immaturity of the lungs, the hemodynamics of infants and animals with congenital diaphragmatic hernia are markedly altered. Hence, cardiac hypoplasia has been implicated as a possible cause of death in patients with congenital diaphragmatic hernia, and it is hypothesized to be a probable consequence of fetal mediastinal compression by the herniated viscera. Cardiac hypoplasia has also been reported in lamb and rat models of congenital diaphragmatic hernia. The purpose of the present experiment was to verify the occurrence of heart hypoplasia in our new model of surgically produced congenital diaphragmatic hernia in fetal rabbits.

**Methods:** Twelve pregnant New Zealand rabbits underwent surgery on gestational day 24 or 25 (normal full gestational time - 31 to 32 days) to create left-sided diaphragmatic hernias in 1 or 2 fetuses per each doe. On gestational day 30, all does again underwent surgery, and the delivered fetuses were weighed and divided into 2 groups: control (non-surgically treated fetuses) (n = 12) and congenital diaphragmatic hernia (n = 9). The hearts were collected, weighed, and submitted for histologic and histomorphometric studies.

**Results:** During necropsy, it was noted that in all congenital diaphragmatic hernia fetuses, the left lobe of the liver herniated throughout the surgically created defect and occupied the left side of the thorax, with the deviation of the heart to the right side, compressing the left lung; consequently, this lung was smaller than the right one. The body weights of the animals were not altered by congenital diaphragmatic hernia, but heart weights were decreased in comparison to control fetuses. The histomorphometric analysis demonstrated that congenital diaphragmatic hernia promoted a significant decrease in the ventricular wall thickness and an increase in the interventricular septum thickness.

**Conclusion:** Heart hypoplasia occurs in a rabbit experimental model of congenital diaphragmatic hernia. This model may be utilized for investigations in therapeutic strategies that aim towards the prevention or the treatment of heart hypoplasia caused by congenital diaphragmatic hernia.

**DESCRIPTORS:** Congenital diaphragmatic hernia. Heart. Hypoplasia. Fetus.

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In previous papers, we have described a new experimental model of congenital diaphragmatic hernia (CDH) in rabbits, and we also have reported on studies of noninvasive therapeutic strategies for prevention of functional and structural immaturity of lungs associated with this defect. These strategies consist of prenatal intra-amniotic administration of porcine sur-

factant or dexamethasone and prenatal tracheal ligation (TL), an invasive method that effectively treats or prevents pulmonary hypoplasia of CDH<sup>1,2</sup>. Based on functional, morphological,

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histomorphometric and biochemical studies, we proved that in this model, CDH is associated with hypoplastic lungs<sup>3</sup>, and the preventive effects of intra-amniotic administration of drugs on lung maturation were comparable to the changes induced by TL<sup>4-6</sup>.

In addition to lung hypoplasia, pulmonary hypertension, biochemical and structural immaturity of CDH lungs,

the hemodynamics of infants and animals with CDH are markedly altered<sup>7,8</sup>. Cardiac insufficiency has also been observed in CDH patients, and it has been suggested that this failure and systemic circulatory problems may be of more importance than pulmonary hypertension<sup>9</sup>. Siebert et al. quantitatively documented cardiac hypoplasia in 8 infants who had died of the complications of left CDH<sup>10</sup>. Consequently, cardiac hypoplasia has been implicated as a possible cause of death in patients with CDH, and it is hypothesized to be a probable consequence of fetal mediastinal compression by the herniated viscera<sup>11</sup>. Cardiac hypoplasia has also been reported in the primary animal models of CDH, i.e. lambs<sup>12</sup> and rats<sup>13</sup>. The purpose of the present experiment is to verify the occurrence of heart hypoplasia in a new model of surgically produced CDH in fetal rabbits.

## MATERIAL AND METHODS

### Experimental Design

Twelve pregnant New Zealand rabbits underwent surgery on gestational day 24 or 25 (normal full gestational time - 31 to 32 days) to create left-sided diaphragmatic hernias in 12 fetuses as previously described<sup>3-5</sup>. Anesthesia was initiated with intramuscular ketamine (45 mg/kg of body weight) and maintained with 0.5% sodium thiobarbiturate intravenously. Briefly, using sterile technique, a maternal laparotomy was performed, and the gravid bicornuate uterus was delivered out of the abdomen. There were usually 6 to 9 fetuses in the uterus of pregnant rabbits. The most distal fetus of each horn was chosen for surgical treatment, and only 1 or 2 fetuses per each doe underwent surgery. During surgical manipulations, the proximal portion of the horn undergoing surgery and the other horn were maintained in-

side the maternal abdomen. The surgical field was continuously moistened with warmed sterile saline solution to prevent fetal hypothermia.

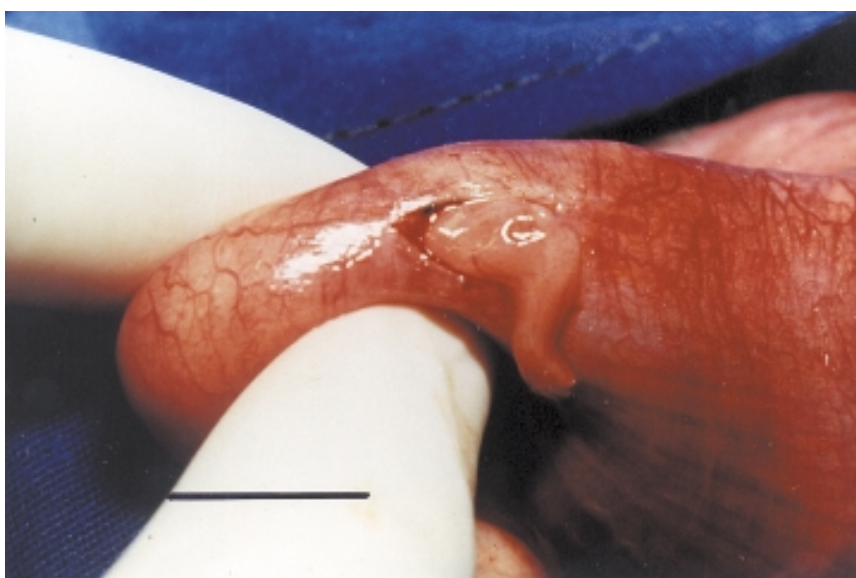
The head and left foreleg of the fetus were palpated, and using microsurgical instruments, a 1 cm transversal hysterotomy was performed at the region overlaying the palpated foreleg. This incision was made as far as possible from the mesometrial border of the uterus. The subjacent chorion and amnion were opened with scissors, and the amniotic fluid was aspirated from the uterus. Only the left foreleg was then exteriorized through the hysterotomy to permit the exposure of the corresponding chest wall (Fig. 1). A low left lateral thoracotomy was performed, and the diaphragm was partially excised by microsurgical scissors. The chest incision was closed in two layers of simple interrupted monofilament 6-0 nylon sutures.

As the hysterotomy was closed with monofilament nylon sutures, warmed sterile saline solution was infused into the amniotic cavity until uterine repletion to reconstitute the amniotic fluid. Finally, the maternal laparotomy was closed. After the sur-

gical manipulations, each doe received 400 mg of cephalothin intravenously and Depo-Provera (5 mg intramuscularly) (NV - Upjohn, Belgium). On gestational day 30, all does were again placed under general anesthesia and the 9 surviving surgically treated fetuses were delivered by cesarean section and sacrificed, body weights were recorded. All the untreated fetuses were also delivered, and 12 of them formed the control group. Through a medial longitudinal incision, the lungs and hearts of all fetuses were collected.

### Histomorphometric studies

The hearts were separated from the great vessels, weighed on a precision balance, and fixed in 10% formalin. Finally, each heart was transversely sectioned in an equatorial plane between the apex and the aortic root, and stained with hematoxylin and eosin. Ventricular wall and interventricular septum thickness were measured by using a Nikon microscope equipped with a 5X magnification objective and a 20X magnification eyepiece that contained a test scale of 1 mm. The pa-



**Figure 1** - Surgical appearance of the left foreleg exteriorized through the hysterotomy (bar corresponds to 1 cm).

thologist, who was masked regarding animal groups, performed at least 20 measurements per each heart.

**Statistical Analysis**

Results were reported as mean ± standard deviation (sd) or mean ± standard error of the mean (sem), and they were compared using analysis of variance. The level of significance was  $P \leq .05$ .

**RESULTS**

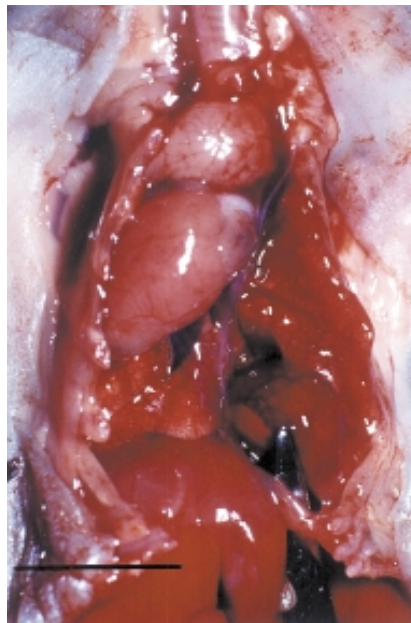
During necropsy, it was noted that in all CDH fetuses, the left lobe of the liver herniated through the surgically created defect and largely occupied the left side of the thorax, with a deviation of the heart to the right side (Fig. 2) and compression over the left lung; consequently, the left lung was smaller than the right one (Fig. 3).

The body weights of the animals were not altered by CDH ( $P > .05$ ) (Fig. 4). However, CDH promoted a significant decrease in heart weights in comparison to control fetuses ( $P < .05$ ) (Fig. 5).

The myocardial structure appeared histologically normal on microscopic examination in control and CDH groups (Figs. 6 and 7), and the histomorphometric studies demonstrated that CDH promoted a significant decrease in the ventricular wall thickness and an increase in the inter-ventricular septum thickness (Tables 1 and 2).

**DISCUSSION**

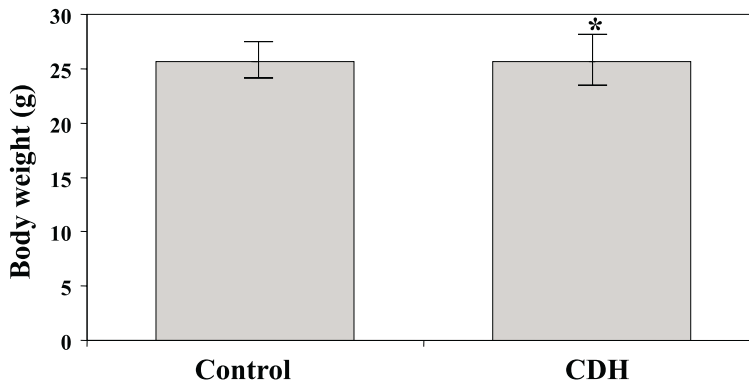
Despite all refinements of the current management, survival rates of patients with CDH remains frustratingly low because of pulmonary hypoplasia, biochemical immaturity of lungs, and arterial thickening and constriction with persistence of the fetal circulation



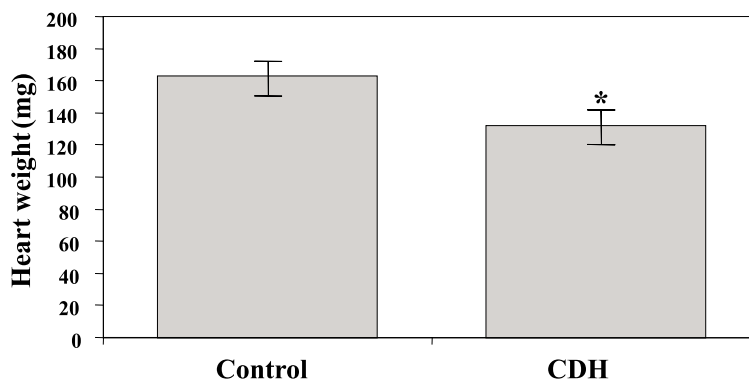
**Figure 2** - Macroscopic appearance of the fetal lungs and heart. Note that the left lobe of the liver is herniated throughout the surgically created defect indicated by the microscissors, compresses the left lung, and the heart is deviated to the right side (bar corresponds to 1 cm).



**Figure 3** - Macroscopic appearance of both lungs and trachea. Note that the left lung is smaller than the right one, due to the compression promoted by the herniated left lobe of the liver (bar corresponds to 1 cm).

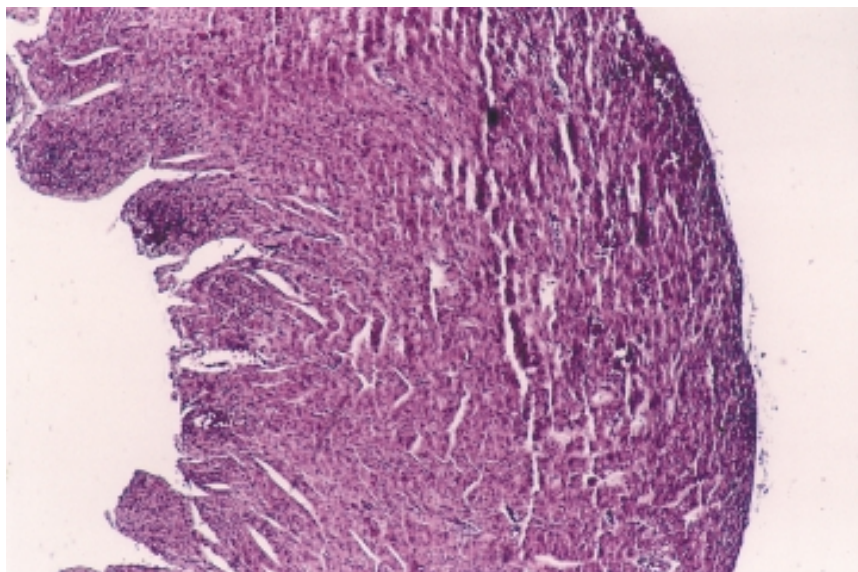


**Figure 4** - Body weights of the fetuses of both groups (mean ± standard error of the mean). CDH did not alter body weights ( $*P > .05$ ).

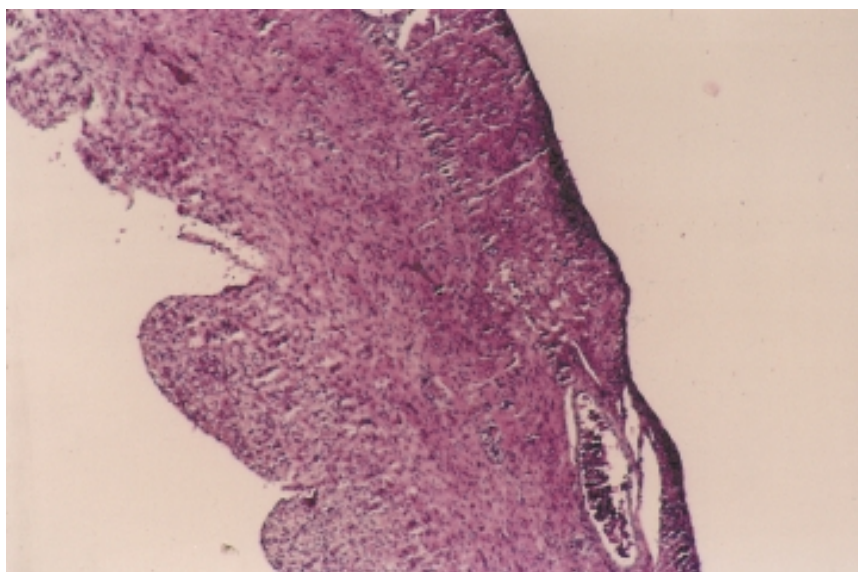


**Figure 5** - Heart weights of the fetuses (mean ± standard error of the mean). Note that CDH promoted a significant decrease of heart weights ( $*P < .05$ ).





**Figure 6** - Microscopic field of ventricular wall from a heart of control newborn rabbit. Note the normal appearance of the myocardial structure (Hematoxylin and eosin, original magnification X100).



**Figure 7** - Microscopic field of ventricular wall from a heart of CDH fetus. Note that the wall is thinner in comparison to a control fetus, showed in Fig. 6 (Hematoxylin and eosin, original magnification X100).

**Table 1** - Ventricular wall thickness (mm).

GROUP	CONTROL	CDH
<b>Ventricular wall (mean ± sd)</b>	(240 measurements)	<b>(400 measurements)</b>
	1.043 ± 0.349	0.888 ± 0.321*

(\*P < .05)

**Table 2** - Interventricular septum thickness (mm).

GROUP	CONTROL	CDH
	(120 measurements)	<b>(200 measurements)</b>
<b>Septum (mean ± sd)</b>	1.054 ± 0.262	1.247 ± 0.409*

(\*P < .05)

pattern<sup>6</sup>. In addition, associated anomalies—primarily cardiac anomalies—account for many of the deaths. The role of hypoplastic heart in the poor prognosis of patients with CDH has been recognized; therefore, hypoplastic heart has been used as an ultrasonographic predictor of poor prognosis in prenatally diagnosed fetuses<sup>14-16</sup>.

The primary focus of the present study was to demonstrate whether the herniated viscera to the thorax produced compression over the developing heart and consequently promoted cardiac hypoplasia. First, since the body weights of the animals were not altered by CDH, we concluded that the decrease in the heart weights promoted by CDH was a clear evidence of heart hypoplasia. Second, the histomorphometric parameters also demonstrated that CDH promoted cardiac hypoplasia. We suggest that the compression of the herniated viscera in CDH and the resulting bilateral pulmonary hypoplasia, as we demonstrated previously in this model, are responsible for the decreased pulmonary blood flow and decreased right ventricular wall thickness. As a result, the decreased pulmonary venous return to the left side of the heart is also responsible for the left ventricular hypoplasia and decreased wall thickness. However we could not explain why CDH promoted an increase in the interventricular septum thickness.

There is some evidence that the compressive mechanism of the herniated viscera to the lungs and heart is the primary reason for the underdevelopment and hypoplasia of both organs, mainly in surgically produced CDH. However, in the nitrofen model of CDH, it was shown that heart hypoplasia was present also in the fetuses without hernia<sup>13</sup>. So we can hypothesize that in that model, other factors are implicated in the hypoplasia other than the anatomic changes or the hypopla-

sia of the lungs and mechanical compression. On the other hand, in the nitrofen model of CDH, the heart-related indices utilized to evaluate left ventricular hypoplasia are not significantly altered, although a global heart hypoplasia is observed<sup>17</sup>.

The reduction in the heart size corresponds to hypoplasia rather than to atrophy, because wet-dry weight and DNA-to-protein ratios were similar in CDH and control groups, according to studies in the lamb model of CDH<sup>18</sup>. Furthermore, it was shown that prenatal dexamethasone rescues in part heart hypoplasia in fetal rats with CDH<sup>19</sup>, and intrauterine correction of surgically created CDH late in gestation reversed

cardiac hypoplasia in the of fetal lamb model<sup>12,20</sup>. However, tracheal ligation in this animal model could not reverse the left ventricular hypoplasia, and this was interpreted as probably caused by the ongoing heart compression promoted by the expanding lung in the tracheal ligated fetus, as we also showed in this rabbit model of CDH<sup>4,5</sup>.

We believe that human CDH and other digestive organ atresias are more complex malformations than the surgically produced CDH, although animal models accurately mimic hypoplasia of both lungs and heart.

In summary, the current study is the first report concerning the deleterious effects of the herniated viscera on the

developing heart in a rabbit model of CDH. Certainly this model may be utilized for future investigations concerning the utilization in the human species of strategies that aim for the prevention or the treatment of heart hypoplasia caused by CDH.

## ACKNOWLEDGMENTS

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## RESUMO

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TANNURI U – Hipoplasia cardíaca em modelo animal de hérnia diafragmática congênita. **Rev. Hosp. Clín. Fac. Med. S. Paulo** 56(6):173-178, 2001.

**Objetivo:** Em trabalhos anteriores investigamos um novo modelo experimental de hérnia diafragmática congênita em coelhos e estudamos também métodos terapêuticos não invasivos para prevenir a imaturidade estrutural e funcional dos pulmões decorrente deste defeito. Além da hipoplasia pulmonar, hipertensão pulmonar, imaturidade bioquímica e estrutural dos pulmões, ocorrem alterações hemodinâmicas significativas em crianças com hérnia diafragmática congênita. Desta forma, hipoplasia cardíaca tem sido implicada como provável causa de óbito em crianças com hérnia diafragmática congênita, e interpretada provavelmente como consequência da compressão exercida pelas vísceras herniadas durante o desenvolvimento do feto. Este fenômeno tem sido rela-

tado também em modelos experimentais de hérnia diafragmática congênita em fetos de ovelhas e ratos. O objetivo da presente experiência é o de verificar a ocorrência de hipoplasia cardíaca em nosso novo modelo de hérnia diafragmática congênita produzida com cirurgia em fetos de coelho.

**Métodos:** Doze coelhas prenhes foram operadas no 24º ou 25º dia de gestação (duração total da gestação – 31 a 32 dias), com o objetivo de produzir hérnia diafragmática esquerda em um ou dois fetos em cada mãe. No 30º dia as coelhas foram novamente operadas para retirada dos fetos, que foram pesados e divididos em dois grupos: controle – fetos não operados (n=12) e grupo com hérnia diafragmática (n=9). Os corações foram retirados, pesados e submetidos a estudos histológicos e histomorfológicos.

**Resultados:** Durante a necropsia verificou-se que em todos os fetos com hérnia diafragmática o lobo esquerdo do fígado sofreu herniação através do defeito produzido cirurgicamente e

ocupou o lado esquerdo do tórax com desvio do coração para a direita, compressão do pulmão esquerdo e em consequência, este pulmão encontrava-se menor do que o direito. O peso total dos animais não sofreu alteração em decorrência da hérnia diafragmática, mas os pesos dos corações estavam diminuídos em comparação aos dos animais do grupo controle. Os estudos histomorfológicos demonstraram que a hérnia diafragmática provocou significativa redução na espessura da parede dos ventrículos e aumento da espessura do septo interventricular.

**Conclusão:** Hipoplasia cardíaca ocorre em modelo de hérnia diafragmática congênita. Este modelo pode ser utilizado em investigações sobre métodos terapêuticos que tenham por objetivo prevenção ou tratamento da hipoplasia cardíaca decorrente da hérnia diafragmática congênita.

**DESCRITORES:** Hérnia diafragmática congênita. Coração. Hipoplasia. Feto.

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