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BASIC SCIENCE ARTICLE

A novel surgical toxicological-free model of diaphragmatic hernia in fetal rats

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BACKGROUND: Teratogen-induced congenital diaphragmatic hernia (CDH) rat models are commonly used to study the pathophysiology. We have created a new and reliable surgically induced diaphragmatic hernia (DH) model to obtain a purely mechanical DH rat model, and avoid the confounding teratogen-induced effects on the lung development.
METHODS: Fetal DH was surgically created on fetuses at E18.5 and harvested at E21.5 in rats. Four groups were evaluated ($n = 16$): control (CONT), control exposed to Nitrofen (CONT NIT), DH surgically created (DH SURG), and CDH Nitrofen (CDH NIT). Body weight, total lung weights, and their ratio (BW, TLW, and TLBR) were compared. Air space (AS), parenchyma (PA), total protein, and DNA contents were measured to verify lung hypoplasia. Medial wall thickness (MWT) of pulmonary arterioles was also analyzed.
RESULTS: DH SURG showed significant hypoplasia (decreased in total protein and DNA) vs CONT ($p < 0.05$); DH SURG vs CDH NIT were similar in TLW and TLBR. DH SURG has less AS than CONT ($p < 0.05$) and similar PA compared to CONT NIT and CDH NIT, MWT were similarly increased in CONT NIT, DH SURG, and CDH NIT.
CONCLUSIONS: This novel surgical model generates fetal lung hypoplasia contributing to the study of the mechanical compression effect on fetal lung development in DH.

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IMPACT:

- There is a critical need to develop a surgical model in rat to complement the findings of the well-known Nitrofen-induced CDH model.
- This experimental study is pioneer and can help to understand better the CDH pathophysiological changes caused by herniated abdominal viscera compression against the lung during the final stage of gestation in CDH fetuses, and also to develop more efficient treatments in near future.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a birth defect characterized by incomplete formation of the diaphragm and migration of the abdominal viscera to the fetal thoracic cavity. This competition for the chest space during critical period of fetal lung development generates pulmonary hypoplasia and histological pruning of lung arteries with pulmonary hypertension. Over the past almost 60 years, CDH animal models have been used to study the pathophysiology of CDH.[1] The CDH animal models can be categorized in congenital (spontaneous in a specific pig breed,[2] genetic modified,[3] and teratogen-induced[4]) or surgically created during gestation.[5,6]

The most commonly used CDH animal model is using the herbicide Nitrofen[4] in rodents with >300 publications reported.[7,8] This model has the advantages of being economical, because is used in small animals, and technically simple, while allowing the study of the lungs and diaphragm embryogenesis mimicking the human diaphragmatic hernia (DH) severe spectrum. However, the Nitrofen model also has disadvantages. First, it is not demonstrated effective in large animals and only described in small rodents. Second, Nitrofen-exposed fetal lungs without CDH are also hypoplastic because the teratogenic effects on multiple organs, particularly affecting multiple lung cell types.[1,7] These major disadvantages confound the study of CDH, as it is impossible to differentiate whether the hypoplasia resulted from the mechanical compression of the hernia contents vs teratogenic effect, or a combination of both.

The surgical models of DH have the advantages as surgically induced DH model is virtually free of confounding systemic effects induced by teratogens and can be performed in large animals for translational studies. However, surgically created models have not been described yet in small rodents and additionally, DH is created

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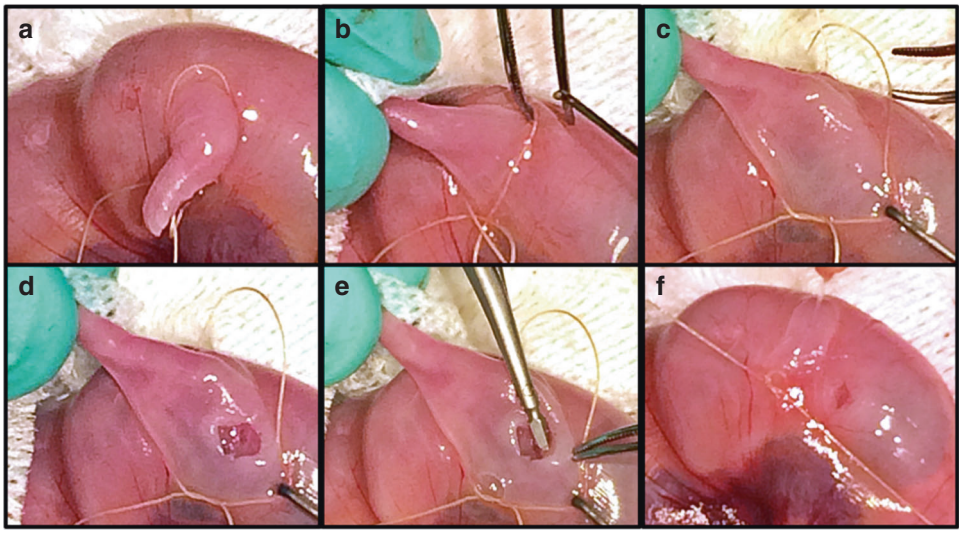

Fig. 1 Surgical creation of CDH. a The left forelimb exposition through a purse suture in the uterine wall at the level of the thorax. **b**, **c** The posterior lateral thoracic wall exposition by gently pulling the left leg. **d** The thoracic incision site—very close to the diaphragm muscle insertion. **e** The scissors insertion into the thorax–abdomen in parallel lateral wall. **f** The purse-string suture closure.

in the pseudo-glandular phase at the earliest, as the procedure becomes 100% fatal if performed earlier. Therefore, surgical models are limited to the study of the fetal lungs from the canalicular stage onward. Despite this limitation, surgical models focus the effect of mechanical compression of the abdominal content on the developing lungs.

To be able to compare the new genetic tools and technologies in the same species, we created a DH surgical model in rats that complements the existing Nitrofen CDH model. This allows the application of newer "omics" techniques to explore mechanistic pathways involved in abnormal lung development in the setting of DH without the confounding effects of teratogens.[9]

METHODS

Ethics in animal experimentation

This experimental protocol was in agreement with the National Institute Guidelines for Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of the Cincinnati Children's Hospital Medical Center—IACUC #2013-0293.

Animals

Sprague–Dawley rats (±250 g; Charles River, Spencerville, OH) were mated and the females were checked daily for vaginal smear. The day of plugging was defined as gestational day (GD) 0 (term = 22 days). The animals were acclimated in controlled thermic environment in a controlled dark-night cycle for 1 week, and received rat specific chow and water ad libitum.

Nitrofen administration

To induce CDH, timed-pregnant rats received 100 mg Nitrofen (2,4-dichloro-4 = -nitrodiphenyl ether, Sigma-Aldrich, Round Rock, Texas) dissolved in 1 mL of olive oil at 9.5 days of gestation by gavage. This dosage of Nitrofen causes 24% of left-sided CDH in the litter.[7,10]

Surgical model

The pregnant rats were anesthetized on day 18.5 of gestation (equivalent to canalicular phase of lung development in human) using a chamber with Isoflurane (Piramal Critical Care Inc., Bethlehem, PA) then placed on a heated water blanket to maintain their body temperature, and they were maintained with Isoflurane set at 2–2.5% and oxygen during the surgery. The abdomen was prepared using Chlorhexidine scrub 2%, and the surgery was executed in sterile conditions.

A median laparotomy was performed from the infra-umbilical area to the pubic symphysis. The bladder and uterus were visualized and one of the uterine horns was exposed. The fetuses were identified by numbers from the cervix to the ovaries, with the fetus closest to the vagina identified as fetus number 1.

The procedure was conducted similarly to the surgical rabbit model.[6] After identification of the fetal position, a purse-string suture was performed in the uterine wall at the level of the thorax using 6.0 Vicryl (Ethicon, Raleigh, NC) suture, and an incision on the uterine wall was made and the fetus anterior left forelimb was exposed through the opening. The thoracic incision was performed gently at the posterior lateral thoracic wall close to the diaphragm muscle with micro scissors pointing to the kidney, in order to perforate the diaphragm. The scissors went into the thorax–abdomen in parallel to the lateral wall and as soon as we felt the diaphragm was perforated, we pulled back with blades open to create a larger defect. The scissors were removed gently from the abdomen and thorax. The thorax remained open and the left forelimb was returned into the amniotic cavity. We added 0.5 mL of warm NaCl 0.9% to refill the amniotic sac, and the purse-string suture was closed (Fig. 1). During the entire procedure the uterine horn was continuously irrigated with warm normal saline. After the DH creation and closure of the uterine wall, the uterine horn was returned to the abdominal cavity and the procedure was repeated on the other uterine horn. The procedure was performed in three to four fetuses per pregnant rat. The pregnant rat abdominal wall was closed in two planes using Vicryl 4.0 suture (Ethicon, Raleigh, NC) for the muscle layer, and Vicryl 4.0 suture (Ethicon, Raleigh, NC) for skin and subcutaneous tissue closure. After surgery, the animals remained isolated with supplemental oxygen and temperature control until they recovered from anesthesia. Later the animals were returned to the animal care facility under the same conditions prior to surgery.

Harvest

The pregnant rats were euthanized at 21.5 days of gestation with CO_2, according to the IACUC protocol. After opening the abdominal cavity, the fetuses were harvested and weighed. A postmortem analysis of the diaphragm integrity was performed.

Experimental groups

A total of 25 pregnant rats were studied and divided in 2 experimental groups: 15 pregnant rats whose fetuses received surgery and 7 pregnant rats whose fetuses received Nitrofen (2,4-dichloro-phenyl-*p*-nitrophenyl ether). Four groups of fetuses were studied ($n = 16$ per group): (1) control (CONT SURG): normal fetuses (siblings from operated mother) not subject to surgery or Nitrofen; (2) control exposed to Nitrofen without development of CDH (CONT NIT); (3) fetus with CDH surgically created (DH SURG); and (4) fetus exposed to Nitrofen who developed CDH (CDH NIT).

The DH SURG group were classified among A, B, C, and D following the CDH group size classification.[11]

Morphological assessment

To evaluate the feasibility of our surgical DH model, we measured the pups body weight (BW), left lung weight, right lung weight (RLW), total lung weight (TLW), and calculated the TLW/BW ratio.

Histological analysis

The left lungs were fixed with formalin, washed in ethanol 70% followed by phosphate buffer, and processed for paraffin embedding. The histological samples were sectioned (5 µm thick) and stained with hematoxylin–eosin (H&E) and Masson trichrome.

Pulmonary air space and parenchyma measurements

H&E-stained lung sections were photographed at 100× magnification in a light microscope and the images were analyzed, using ImageJ2 (National Institutes of Health, Bethesda, MD). The air space (AS) was measured by subtraction of total area minus parenchyma (PA) area. Quantification was conducted using more than ten random images in two consecutive slides from four fetuses per group. The results were expressed in pixel/µm.

Vascular measurements

Masson trichrome stained lung sections were analyzed under 200× magnification. Pre-acinar resistance arterioles between 30 and 60 µm were included. We measured the external diameter (ED), internal diameter (ID), and calculated the proportional medial wall thickness (MWT) with the formula: MWT = (ED − ID)/ED. This formula eliminates the effects of vasodilation, vasoconstriction, or changes caused by the tissue fixation over the morphometric variables.[12] Quantification was conducted using 40 random vascular arterioles' images in two consecutive slides from four different fetuses per group by blinded investigators, using the software Image Pro Plus 6.0 software (Media Cybernetics Inc., Rockville, MD).

Total protein contents

Total proteins were extracted from snap-frozen lungs ($n = 4$ fetuses in each group) by sonication homogenization (Fisher Scientific, Pittsburgh, PA), using T-PER (Thermo Fisher Scientific, Rockford, IL) + proteinase K + phospho STOP (Roche Diagnostics GmbH, Mannheim, Germany) buffer in 1.5 mL tubes, then the homogenate was centrifuged at 10,000 r.p.m. for 5 min. Protein concentration was assessed by the BCA-mini method (Thermo Scientific, Rockford, IL). The concentration of protein per lung was accessed by the ratio: total protein per TLW in mg.

Total DNA contents

Snap-frozen lungs ($n = 4$ fetuses in each group) were homogenized, and the total DNA of each lung was extracted using a commercially available kit (DNeasy® Blood and Tissue Kit; Qiagen, Hilden, Germany). The total DNA content was measured using a spectrophotometer (Epoch Microplate Spectrophotometer, Biotek, Winooski, VT).

Statistical analysis

All statistical analysis and graphs were performed in GraphPad Prism 9 software (GraphPad Software Inc., La Jolla, CA). The differences among multiple groups were analyzed by one-way analysis of variances, using Turkey's post hoc test. The results are described as means ± standard deviation for all morphometric, PA, AS, arterioles, protein, and DNA analysis. A p value < 0.05 was considered statistically significant.

RESULTS

General

DH surgically created. A total of 15 pregnant rats were submitted to surgery in order to create fetal DH and a total of 70 fetuses out of 132 had DH surgically created (53%). The average of performed surgery per pregnant rat was less than four. After DH surgery, 28 fetuses died (40%) and 42 survived (60%), 12 out 42 (28.6%) do not have a hole at the diaphragm muscle (failure of CDH), 14 out of 42 (33.3%) had a small (type A) or medium size (type B), not considered for the model analysis, and 16 out 42 (38.1%) had a large defect (type C) and complete defect (type D) DH, according to CDH Group classification.

Table 1. General results of surgeries with survival rate between CDH SURG and CDH NIT.

	DH SURG	CDH NIT
Rats	15	7
DH surgeries per rat	3–4	NA
Total of fetuses	132	58
DH fetal surgery	70 (53%)	NA
Dead	28 (40%)	0
Survival	42 (60%)	58 (100%)
No hole (failure)	12 (28.6%)	42 (73%) exposed
DH types A and B	14 (33.3%)	0
DH types C and D	16 (38.1%)	16 (27%)

NA not applicable.

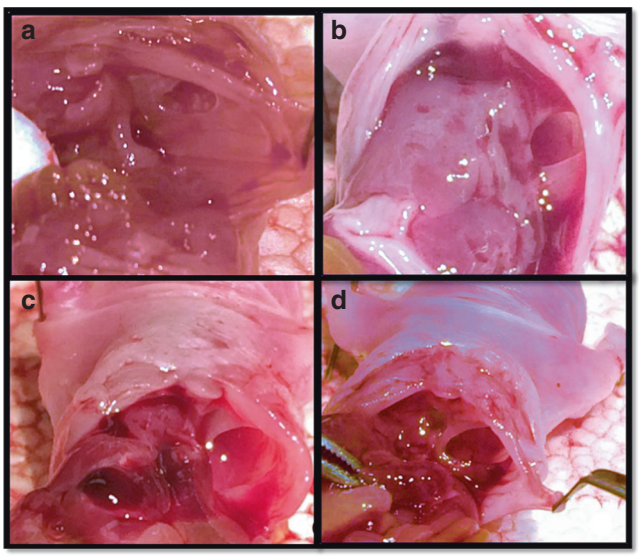

Fig. 2 Surgical DH classification according to hole size of DH Study Group. a Defect A. **b** Defect B. **c** Defect C, and **d** defect D.

CDH Nitrofen model. A total of 7 pregnant rats were gavaged with Nitrofen, and a total of 58 fetuses were exposed to the teratogen agent. At delivery, 42 out 58 (73%) had no CDH and 16 (23%) had left severe CDH.

Comparison between fetuses with DH surgically created (DH SURG) and fetuses exposed to Nitrofen with CDH (CDH NIT) is shown in Table 1, the size of holes ranged from CDH C and D[11] (Fig. 2).

Morphological assessment

CDH characterizes by the herniation of abdominal organs through the defect into the thoracic cavity causing compression of the lungs and lung hypoplasia with 50% fewer bronchiole generations. To study these alterations in DH animal models, we first weight the fetuses and the lungs. As expected, Nitrofen-exposed animals (CDH NIT) and DH SURG showed less BW than control fetuses (**$p < 0.005$; Table 2 and Fig. 3). When comparing the TLW of Nitrofen CDH fetuses showed less weight than control as predictable, but also Nitrofen control animals and our surgical created DH showed less lung weight than control lungs (**$p < 0.005$; Table 2 and Fig. 3). If analyzed each side lung separately, we observed that Nitrofen CDH has affected the weight of both lungs, while surgical DH only the left side where the hole was created (*$p < 0.05$, **$p < 0.005$). Both DH animal models showed less lung/BW ratio (**$p < 0.005$; Table 2 and

Table 2. Morphometrics results among the four groups.

	CONT ($n = 16$)	CONT NIT ($n = 16$)	DH SURG ($n = 16$)	CDH NIT ($n = 16$)	p
Body weight (mg)	5685 (±753)[a]	3434 (±196)	4750 (±521)[b]	3654 (±235)	$p < 0.005$[a,b]
Right lung weight (mg)	87 (±2)[a]	70 (±8)	71 (±16)[c]	58 (±9)	$p < 0.005$[a], <0.005[c]
Left lung weight (mg)	49 (±7)[a]	34 (±6)	36 (±5)[c]	26 (±6)	$p < 0.005$[a], <0.005[c]
Total lung weight (mg)	159 (±23)[a]	104 (±12)	98 (±14)	84 (±13)	$p < 0.005$[a]
Lung to body ratio (%)	0.030 (±0.006)[d]	0.031 (±0.004)	0.021 (± 0.003)	0.023 (± 0.004)	$p < 0.005$[d]

[a]CONT > DH SURG > CONT NIT and CDH NIT**.
[b]DH SURG > CONT NIT and CDH NIT**.
[c]DH SURG > CDH NIT*.
[d]CONT = CONT NIT >DH SURG and CDH NIT** (*$p < 0.05$ and **$p < 0.005$).

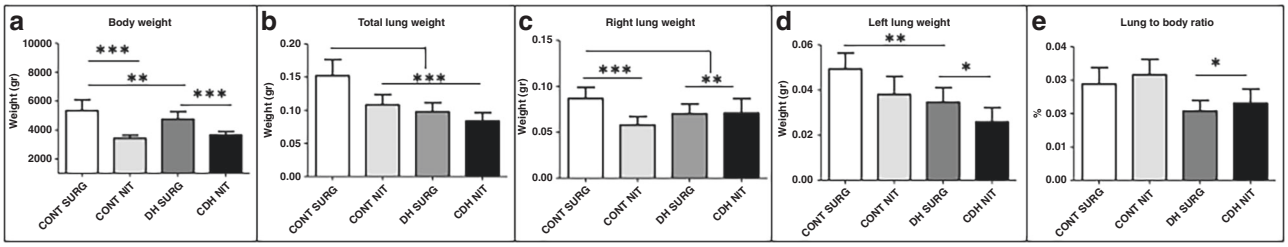

Fig. 3 Graphics of morphological analysis. a Body weight. **b** Right lung weight. **c** Left lung weight. **d** Total lung weight. **e** Total lung to body ratio. *$p < 0.05$, **$p < 0.005$. ***$p < 0.001$.

Fig. 3) than controls. DH SURG showed significant hypoplasia vs CONT (*$p < 0.05$, **$p < 0.005$; Table 2 and Fig. 3).

Pulmonary parenchyma and air space measurements

Next, we examin ed the PA in both animal models. Nitrofen-exposed animals and surgical DH exhibit increased PA density with less ASs compared with control animals (**$p < 0.005$; Fig. 4). No PA differences comparing DH SURG to Nitrofen CDH and control Nitrofen, but CDH Nitrofen has less AS than surgical DH animal model (*$p < 0.05$ **$p < 0.005$; Fig. 4).

Vascular measurements

The vascular defects associated with pulmonary hypertension in CDH, which is characterized by increased muscularization of arterioles and capillaries. When comparing the wall thickness of the arterioles in the DH models, we observed that the average of pulmonary arteries medial wall thickness in pulmonary arterioles (MWT) were increased in Nitrofen-exposed animals CONT NIT and CDH NIT (**$p < 0.005$; Fig. 5). The surgically created rat model DH SURG also showed higher wall thickness when compared to control fetuses (**$p < 0.005$; Fig. 5).

Total protein contents and DNA contents

Total protein and total DNA content was measured in the lungs to assess the pulmonary growth and size. DH fetuses surgical DH (CDH SURG) and CDH Nitrofen (CDH NIT) showed less total lung protein and less total DNA content (*$p < 0.05$; Fig. 6) than controls. Nitrofen-exposed control (CONT NIT) also had a decreased total lung protein and DNA content when compared to control animals (CONT; *$p < 0.05$; Fig. 6). We did not find any significant differences in the total protein or DNA between CONT NIT, DH SURG, and CDH NIT (Fig. 6).

DISCUSSION

Animal models are required and used to advance in CDH research. The teratogenic model has been employed in rats, while surgical models have been commonly used in larger animals, such as sheep, and more recently, also described in rabbits.[6] These models each have their unique advantages and limitations as they do not perfectly replicate the congenital disease process. Also, data obtained from one model is many times not complementary to others due to interspecies differences.[10]

The teratogenic model has been widely used to study the pathophysiology of CDH, helping to understand abnormal lung and vascular development and the origin of the diaphragmatic defect formation.[12] Although the teratogenic effects of herbicide Nitrofen in rodents was described in the 70s[4], it was rediscovered in the 80s (refs. [13,14]) with an increase in number of publications in the 90s.[7,8,15]

The exact administration time and the right dose necessary to create CDH has been studied over the years, yielding variable rates of CDH incidence. When Nitrofen is given on GD 9.5, left-sided CDH is seen in 25% to 40–60% of the offspring. However, when Nitrofen is administered on GD 11, right-sided CDH is more commonly produced.[7,8] Since Nitrofen is administered during lung organogenesis, it is a better model to study the embryogenesis of CDH compared to the surgically created alternative.

Importantly, Nitrofen-exposed rat fetuses that do not develop a diaphragmatic defect, still demonstrate significant lung hypoplasia, possibly due to a toxic drug effect. Strong evidence suggests that Nitrofen interferes with the retinoid signaling pathway, which may explain the teratogenic etiology of CDH.[16] Teratogenic lung hypoplasia in the absence of a diaphragmatic defect is a major confounding limitation of the Nitrofen model when we want to focus our study in the mechanical lung compression effect.[17] Also, Nitrofen produces agenesis of the left hemidiaphragm resulting in very large defects that does not reflect the size spectrum of defects seen in human CDH cases.[18] Contrarily, this last point is achieved by the surgical model that produces all the spectrum of severity from small to large diaphragmatic defects either in the selected right or left side.

The surgically created DH model has the advantage of essentially isolating the compressive mechanical effect of herniated abdominal viscera on the lungs during the canalicular phase of lung development. Surgically created models are also useful to study the impact of fetal surgical interventions on lung development and pulmonary hypertension.[19] However, the surgical model cannot be

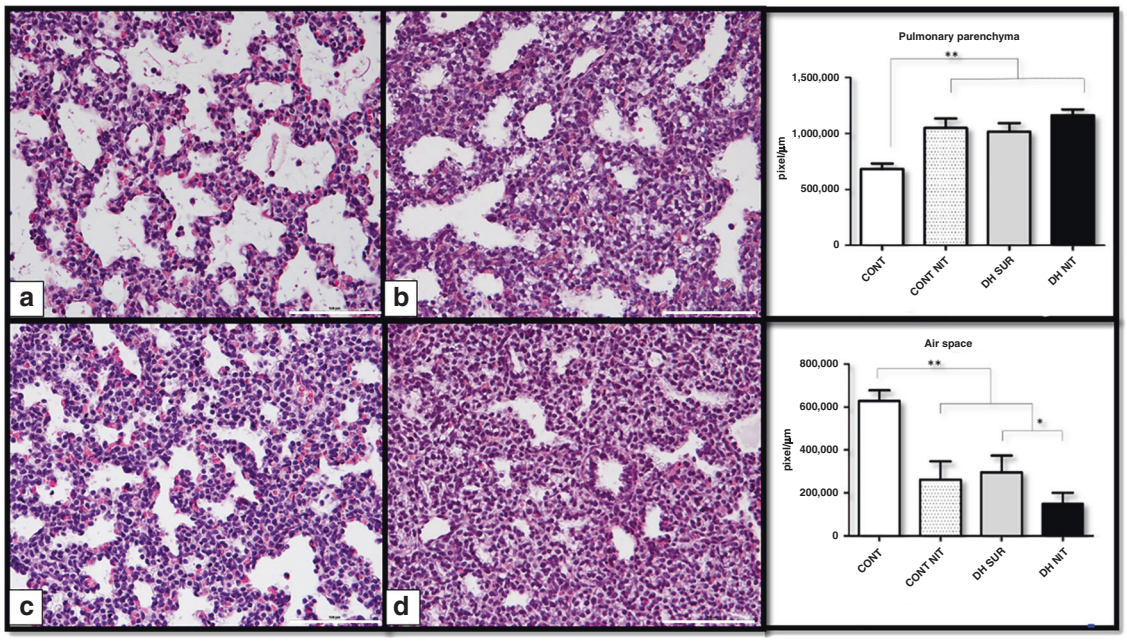

Fig. 4 H&E stain of pulmonary parenchyma. a Control group (CONT). **b** Control Nitrofen group (CONT NIT). **c** CDH surgical group (CDH SURG). **d** CDH Nitrofen group (CDH NIT). Magnification: 100×. Scale bar: 100 µm. *$p < 0.05$, **$p < 0.005$.

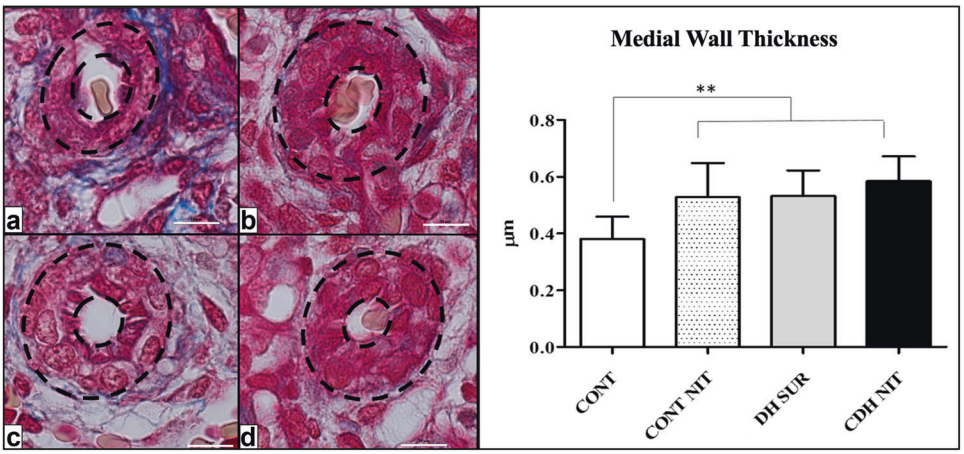

Fig. 5 Pulmonary arteries wall thickness. Average of pulmonary arteries medial wall thickness in the different groups. Photomicrographs of pulmonary arterioles stained with Masson's trichrome. **a** Control group. **b** Control Nitrofen group. **c** CDH surgical group. **d** CDH Nitrofen group. Magnification: 400×. Scale bar: 50 µm. **$p < 0.005$.

used to study the early embryologic stages of lung development because the surgical induction is performed during the pseudo-glandular or canalicular stages. Therefore, surgical models can only be used to study the lungs from the canalicular stage onward.[20]

The first surgical model of DH was performed in sheep fetuses.[5] The authors created the DH defect by opening the fetus' chest in a left intercostal space, removing part of the diaphragm muscle in the third trimester of gestation during the pseudo-glandular phase of lung development. The analysis included a comparison of lung size and volume with the gestational age at defect creation (from day 98 until day 138, term of gestation = 145–150 days). The harvest was performed at term and the results showed a decrease in the lung weight from 25 to 75%, trending higher in fetuses where surgery was performed earlier in gestation. There was associated smaller AS and pulmonary hypoplasia, which was more severe also in cases with earlier surgical DH creation.[5]

A less invasive technique has been described by placing an inflatable balloon into the left fetal hemithorax.[21] The mechanical effects of increased intrathoracic pressure cause reversal of ductal flow and decreased perfusion of the ipsilateral lung. A similar model trying to emulate the in utero correction of DH,[22] used higher insufflation volume (60–150 mL), followed by deflation of the balloon on day 120 of gestation. They found a reversal of lung hypoplasia with an increase in lung weight, air capacity, compliance, and area of the pulmonary vascular bed.[22] Another study found a decrease in pulmonary vasculature that correlated with the time of the hernia creation, and the fetal DH defect correction reversed the histological findings of DH.[23]

The surgical DH sheep model is now standardized and the diaphragmatic defect is performed between days 60 and 63 of gestation[23] (pseudo-glandular phase of lung development). The main advantage of the sheep model is the larger fetal size allowing for easier diaphragmatic defect creation. The

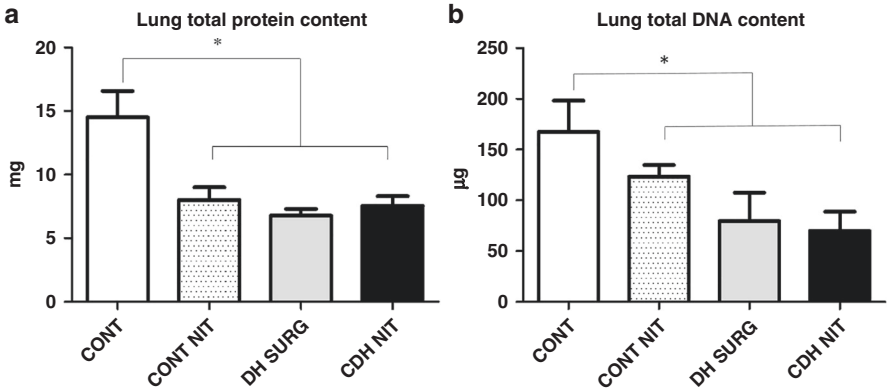

Fig. 6 Total protein and DNA per group. a Lung total protein content. **b** Lung total DNA content. *$p < 0.05$.

disadvantages include the cost and space of maintaining sheep, few fetuses per pregnancy, and a long gestational period to maintain these animals.

The first surgical model of DH performed in rabbit fetuses[24] at 23–26 days (pseudo-glandular phase of lung development; term = 31–32 days) reported a high mortality rate (>45%) and no significant pulmonary hypoplasia. Another group attempted the DH creation on day 20 of gestation with 100% mortality.[6] The same group then tried diaphragm defect creation on days 24 and 25 of gestation, which led to a 70% fetal survival rate with significant pulmonary hypoplasia and increased pulmonary vasculature thickness.[6] A third group performed the surgery on day 23 of gestation and the fetuses were harvested on days 25, 27, 29, and 30 of gestation, describing changes compatible with pulmonary hypoplasia and progression over time.[25] Recently, a whole DH lung transcriptome in the surgical DH rabbit model and fetuses with tracheal occlusion (TO) has been published, in order to better understand the cellular pathways involved in the pulmonary hypoplasia and its improvement by TO.[26]

The advantages of the rabbit model include lower cost and a shorter gestation than sheep, with greater number of fetuses,[24] but there is not a teratogenic rabbit model available to study the earlier phases of lung development to complement the surgical model.

In this report, we aimed to create a new surgically created DH model in rat to fulfill the gaps and limitations presented by other induced DH models. Our surgically created DH model in fetal rats has a tolerable mortality and results in lung hypoplasia and increased pulmonary arteriole medial wall thickness comparable to other surgically created models, such as sheep and rabbit. The technique is simple and survival is minimally influenced by learning curve in skilled hands. The lung hypoplasia in the DH SURG group was more pronounced than in the CONT NIT and CONT groups. When compared to the CDH NIT, lung hypoplasia was less pronounced in the DH SURG group, but this finding is likely due to the severity and combined effects of compression and possible direct teratogenicity expected in the Nitrofen model.

To our knowledge, this is the first surgical model of DH described in rats. Our results show a lung–BW ratio similar to the Nitrofen CDH model; however, our surgical created DH RLW was higher when compared with the Nitrofen CDH RLW, either in our results and presented in previous papers.[7,15] This can be the result of a later insult in the surgical DH model as compared with the Nitrofen, and also the constant presence of liver-up and severe type D diaphragmatic defect in the Nitrofen model. The RLW is lower than the control so we can still assume a certain degree of hypoplasia (Table 2 and Fig. 3), but less than seen in the Nitrofen model. In addition to hypoplasia, we histologically observed a reduction of AS, an increase in PA (Fig. 4), and an increase in the medial wall thickness of the pulmonary arterioles (Fig. 5), all similar to findings in the Nitrofen model.[27,28] Furthermore, the amount of protein and DNA was decreased in the surgically created DH model similar to the Nitrofen CDH model,[29,30] corroborating the lung hypoplasia in our model.

Another advantage of this rat surgical model is the full spectrum of DH size defects, like those found in humans. As we mentioned, Nitrofen model mainly generates large diaphragmatic defects equivalent to sizes C and D. We selected the larger defects created by surgery for this particular study just to be comparable with the ones obtained by the teratogen. Study of lung histology and effects of compression in smaller DH surgical defects could be focus of another studies.

Although the use of animal models has inherent limitations, it is important to complement information between different models preferably in the same animal species. This can deeply increase our knowledge about the lung and diaphragm development in CDH. Moreover, many pathways, previously inaccessible for scientific inquiry, could now be investigated. Conversely, not all genes altered in these animal models are found to cause CDH in humans.[19]

The limitations of our study include size, time, and surgical trauma. The diaphragmatic defect creation requires training in microsurgery. The surgical procedure is performed only after the pseudo-glandular phase of lung development, making this model not useful for studying lung development before this stage. Lastly, the surgical intervention could cause trauma and inflammation in the tissue, which should be considered when the data are analyzed.

Our model, in the other hand, offers the possibility to use of "omics" technologies in the canalicular stage of lung development without the confounding effects from a teratogenic model.

CONCLUSION

This novel surgical model is comparable to Nitrofen CDH in generating pulmonary hypoplasia and all the pulmonary histological features of CDH, while avoiding confounding teratogenic effects, and can be useful to study the mechanical compression effect on fetal lung development. We found out that a surgically created DH model in rats is feasible and reproducible. It is possible to achieve a significant diaphragmatic defect, showing a spectrum of DH sizes, on the right or left side, and obtain histological changes of PA and pulmonary arterioles similar to the Nitrofen model. A surgical model devoid of teratogens creates an opportunity to apply genomics and transcriptomes analyses of the canalicular phase of lung development to advance the study of DH, and complement the widely used Nitrofen model.

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AUTHOR CONTRIBUTIONS

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COMPETING INTERESTS

The authors declare no competing interests.

CONSENT STATEMENT

Patient consent was not required.

ADDITIONAL INFORMATION

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