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Fetoscopic abdominal decompression of congenital diaphragmatic hernia – a proof of concept study and stereological analysis based on morphological pulmonary changes in an ovine animal model

Dissertation

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Introduction

A congenital diaphragmatic hernia (CDH) is a malformation in which abdominal viscera prolapse during fetal development through an opening in the diaphragm into the thoracic cavity. As a result, intrathoracic pressure increases, hindering a physiological development of the fetal lung causing lung hypoplasia and consequent pulmonary hypertension (Montalva et al. 2019).

Epidemiology

It is estimated that the incidence of a child born with CDH lies at around 1 in 2,200 live births (Langham et al. 1996). It is important to note that many pregnancies are terminated if a CDH is diagnosed in the child. The true incidence is therefore suspected to be higher (Harrison et al. 1978).

Statistically, 85% of all CDH malformations occur on the left side and are known as Bochdalek hernia. Another 10% occur on the right side (Morgagni type) while only 5% occur on both sides. Forty per cent of CDH cases occur in combination with other malformations. Fetuses with Morgagni hernias are much more likely to develop septal-heart defects, face malformations, and malformations in their extremities compared with fetuses with only a left-sided CDH (Langham et al. 1996). In addition, there is a high comorbidity with other genetic syndromes such as Down syndrome, Fryns syndrome or Cornelia de Lange syndrome. The other 60% are isolated CDHs with no other malformations or chromosomal aberration (Slavotinek et al. 2014).

Pathophysiology

The formation of the diaphragm occurs in around the fifth to eighth embryological week. The pleuroperitoneal channel is separated by an infolding membrane of the corporal wall, followed by fusion of lateral and central segments with esophageal mesentery cells. This is followed by invasion of dermomyotome cervical somites,

forming the muscular plate of the diaphragm (Stevenson et al. 2006, Marlow and Thomas 2013). Embryological formation of the diaphragm overlaps with an important phase of fetal lung development (Wigglesworth et al. 1981). As such, when abdominal viscera prolapse into the thoracic cavity, compression is exerted on the developing lungs. Children born consequently present hypoplastic lungs, characterized by a reduced amount of alveolar volume, thickened septa and a low amount of bronchioles (Mühlfeld et al. 2013). It is believed for this pathophysiology that neonates face respiratory failure, ventilation dysfunctions or persistent pulmonary hypertension, which, depending on its severity, is considered to be the most important determinant of neonatal morbidity (Seetharamaiah et al. 2009).

Clinical manifestation

Common complications infants face after birth are respiratory associated infections, and the development of pulmonary hypertension (Montalva et al. 2019). In cases of severe lung hypoplasia, it is possible for primitive alveoli to rupture, causing an additional pneumothorax. Another major complication posing additional difficulties in postnatal therapy and neonatal survival of CDH arises if the liver is elevated and displaced into the thoracic cavity. A differently positioned liver may cause an abnormal vasculogenesis, which can lead to the decline of the organ during the attempt of surgical repositioning (Speer and Gahr 2012).

Although significant advances have been made with the optimization and evolution of modern neonatal care, CDH still remains a critical condition (Snoek et al. 2015). The overall survival rate of CDH in newborns is considered around 70% (Stevenson et al. 2007). However, this figure misinterprets the true severity of the disease. In fact, many authors have shown that if CDH is categorized in severity, ranging from mild to extreme, survival rates quickly diverge between 90% for mild cases to less than 10% for extreme or severe cases (Deprest and De Coppi 2011).

Prediction of neonatal survival

A general prediction can be made by estimation of lung size by 2D ultrasound or fetal MRI, which can approximate the level of expected lung hypoplasia and consequential pulmonary hypertension. The most accurate prediction can be made by measurement of the lung to head ratio (LHR) as a function to gestational age and change over time expressed as the observed/expected LHR (Mullassery et al. 2010). Figure 2 illustrates how applying the O/E (observed to expected) LHR is suitable for categorizing the severity of CDH and making a prediction on the survival rate.

By evaluating Figure 2, it becomes apparent that the overall survival rate remains dismal in severe to extreme cases of CDH, even with modern postnatal therapy. Research has thus focused on prenatal interventions, hoping to treat the defect before birth, in order to minimize subsequent damage and prevent irreversible pulmonary hypoplasia and hypertension (Chen et al. 2009, Style 2019).

Current treatment and fetoscopic interventions

Modern management of congenital diaphragmatic hernia varies between neonatal tertiary centers. A CDH EURO consortium was established in 2008 by the leading experts in the field of CDH in order to create evidence-based guidelines for clinicians and improve the overall survival outcome of newborns with CDH. These recommendations include guidelines for the treatment in the delivery room, treatment on the neonatal intensive care unit, the treatment of pulmonary hypertension, the use of ECMO and lastly on optimal surgical repair. Examples of such recommendations include immediate intubation after birth, obtaining preductal oxygen saturation between 85% and 95%, and the use of inhaled nitric oxide for pulmonary hypertension (Snoek et al. 2016).

It should be noted that all these interventions do not reverse the underdevelopment of the fetal lung with the resulting pulmonary hypertension. As such, approaches were developed to intervene prenatally, in order to optimize fetal lung development and reduce consequential pulmonary hypertension (Kahn et al. 2007).

The most important development in prenatal intervention of CDH was made with the establishment of fetoscopic endotracheal occlusion (FETO), a method in which a balloon is inserted in the trachea of the fetus, causing intratracheal fluid pressure to increase, and consequently the stimulation of cell proliferation and maturation of pulmonary vessels (Khan et al. 2007, Van der Veeken et al. 2018, Style et al. 2019). A recently published study conducted by Style et al. (2019) presents further evidence that besides improving survival in moderate to severe CDH cases, FETO also significantly improves the severity of long-term pulmonary hypertensions in infants (Style et al. 2019).

To date, FETO is the only routinely, yet still experimental, practiced fetal surgical intervention in the treatment of CDH (Deprest et al. 2011, Van der Veeken et al. 2018). First trials in the ligation and occlusion of the fetal trachea were conducted in 1998 by Harrison et al. in San Francisco. Since then, the surgical techniques have been refined and optimized. Further conducted trials by Harrison et al., the FETO consortium, and a South American series showed that FETO increased survival in cases of severe CDH (with liver up) to up to 40% compared with survival rates of under 10% in cases of severe CDH without intervention (Peralta et al. 2011). Further promising results have been published by the Mayo Clinic, which reports on two successfully treated CDH cases with no major complications (Ruano et al. 2018).

The first substantial piece of evidence for the effectiveness of FETO was presented in Jani et al.'s 2009 published paper "Severe diaphragmatic hernia treated by endoscopic tracheal occlusion". It showed that FETO worked and improved neonatal outcome (Jani et al. 2009). The study included a total of 210 CDH cases. Some 175 were left-sided hernias, 34 right-sided and one bilateral. A total of 188 cases had an isolated CDH while the other 22 were associated with other congenital malformations. Insertion of a silicon-rubber balloon was carried out at a median gestational age of 27.1 weeks. Major complications of the intervention included spontaneous preterm pre-labor rupture of membranes (PPROM) in 99 cases (47.1%). The technical success of FETO was very high at 96.7% (203 cases). However, only 98 (48%) of newborns survived overall. In order to compare the effectiveness of the intervention, the authors matched the individual cases with the expected survival, calculated from the O/E LHR. Using

these figures, survival increased in left-sided CDH from 24.1% to 49.1% and in right-sided CDH from 0% to 35.3% ($p<0.001$). Jani et al.'s paper includes the largest number of cases of fetuses with CDH treated with FETO to date. Its weakness is its lack of control groups and randomization. As such, these promising results have to be treated with caution. Similar results were also observed in trials conducted in Brazil (Peralta et al. 2011) and Germany (Kohl et al. 2006).

The first randomized controlled trial comparing the effectiveness of FETO with conventional postnatal management in cases of severe CDH was published in 2012 by Ruano et al. Between 2008 and 2010 the authors included 41 patients in their trial. Entry criteria were an LHR <1.0, a liver herniation and no other congenital defects. Twenty fetuses were randomly allocated to the FETO group and 21 to the control group. Similarly to Jani et al., the tracheal balloon was placed between the 26th and 30th week via fetoscopy. The paper concluded that ten out of 20 (50%) newborns survived in the FETO group compared to a survival rate of one out of 21 (4.8%) in the control group. The relative risk was 10.5 (95% CI, 1.5-74.7) with $p<0.01$. Although the number of cases is small, the authors verify the reported effectiveness of FETO in Jani et al.'s work.

Even though these studies show promising results, evidence is still far away from recommending FETO as a standard intervention (Deprest et al. 2011). The ongoing "TOTAL – Tracheal Occlusion To Accelerate Lung Growth" project (funded by the European Union and organized by many experts from the CDH EURO consortium) is the first global double-blinded prospective multicenter study. It will compare the effectiveness of FETO for cases of light, moderate and severe CDH. The final evaluation of FETO will therefore only be possible after publication of the first results of the TOTAL trial, which are expected to be published between 2020 and 2022 (htttps://www.totaltrial.eu) (Dekoninck et al. 2011).

Alternative approaches in fetal surgery in the management of CDH

FETO interventions have greatly contributed to improved survival in cases of severe CDH (Deprest et al. 2011). However, the problem of the intestine prolapsing into the thoracic cavity remains. The pressure exerted by the hernia on the developing lung

remains one of the main causes of the resulting lung hypoplasia, as experimentally demonstrated by Harrison et al. (1980).

In his study a conical silicone-rubber balloon was inserted in six fetal lambs and progressively inflated to mimic a developing CDH (Harrison et al. 1980). All six lambs died due to respiratory insufficiency despite resuscitation efforts. Five other lambs also received a balloon, which was gradually inflated, however this balloon was deflated at day 120 to simulate a “corrected hernia”. All five lambs survived and showed improved values in terms of lung volume, compliance and area of pulmonary vascular bed. Naturally this experiment cannot be compared to a large multicenter trial with greater case numbers and larger control groups. It does however reveal a fundamental aspect of CDH: an intrathoracic pressure increase can increase the amount of dysfunctional lung parenchyma. The question is, does pressure reduction improve lung development (Porreco et al. 1994)?

Resulting from the basic work laid out by Harrison et al., research also looked into the possibilities of reducing intrathoracic pressure prenatally and improve survival outcome and lung quality. An interesting hypothesis was formulated by Zaupa et al.’s case report “Bilateral congenital diaphragmatic hernia and gastroschisis in a newborn: can low intrathoracic pressure prevent the pulmonary hypoplasia?”. The authors describe the case of an infant born at 38 weeks by a 28-year-old woman with bilateral CDH and gastroschisis. Yet, in contrast to what might be expected, the infant showed a much better outcome than other newborns with bilateral CDH. Furthermore, there was no evidence of relevant lung hypoplasia. Hence, Zaupa raises the question of whether the presence of gastroschisis decreases the intrathoracic pressure resulting in the good (Zaupa et al. 2007) outcome.

The idea of thoracic pressure release through gastroschisis is not new. In a somewhat radical approach Porreco et al. published in 1994 a case report entitled “Palliative fetal surgery for diaphragmatic hernia” in which they described iatrogenic gastroschisis in a fetus with CDH. According to the report, fetoscopic abdominal decompression (fAD) was established through open fetal surgery (hysterotomy) during the 22nd week of pregnancy. The neonate was delivered at 28 weeks and experienced various complications (necrotizing enterocolitis, retinopathy, and respiratory distress requiring

surfactant therapy and ventilation). Unfortunately, the case report did not record what the O/E LHR of the fetus was, nor did it include any follow-up data. In a letter to the editors published a year later, others correctly criticized the approach for its lack of scientific method (especially the lack of animal testing), and the approach of open fetal surgery. Despite all the criticism, Porreco et al. did show that fAD could offer new ways in the treatment of CDH.

First substantial animal studies investigating the possibility of fAD in the treatment of CDH were conducted by Chun et al. 2009 and Chen et al. 2008, who investigated lung hypoplasia in a rabbit model. Montgomery was the first who investigated the possibility of fAD in the more complex animal model of sheep. Even though the results showed a high mortality from the procedure (90%), they were able to demonstrate that abdominal decompression could be performed in more complex animal models. It should be noted, however, that the results were carried out by open fetal surgery. This is a very important factor, as open fetal surgery is associated with a higher rate of preterm birth, a larger amount of fetal trauma and a lower rate of neonatal survival (Luks et al. 2011).

Aim and hypothesis

It becomes apparent that current research concerning abdominal decompression in CDH is lacking in fetoscopic approaches. As seen with tracheal occlusion, and with many other fetal interventions, the less radical the surgical approach the higher the fetal survival and the lower the maternal complications are (Deprest et al. 2004, Luks et al. 2011).

In previous studies abdominal decompression for CDH was performed by open fetal surgery, a factor which greatly contributes to a higher mortality. As such, transference of any of those methods onto humans would bare too many risks for the mother and her unborn child. Therefore, the idea arose to create a minimally invasive (fetoscopic) procedure for abdominal decompression in an ovine CDH model.

The aim of this study is to establish a technically successful de novo proof of concept model for fetoscopic abdominal decompression in fetal sheep with a surgically created CDH. It is hypothesized that morphological changes, indicative of parenchymal

change, will occur in CDH lungs undergoing fAD. Analysis of pulmonary structural changes will be estimated via lung stereology. The method should allow an “unbiased and efficient estimation of structural features without making any assumptions on the underlying nature of the biological sample” (Mühlfeld et al. 2013).

Materials and methods

Research design

The research design and the permission to use an animal model was approved by the State Administration for Animal Research under the name “project 78/12 – FETAL-CDH I” and conducted under the rigorous surveillance of the institutional animal care and use committee. Twelve German blackhead ewes (Ovis aries) were approved and allocated to the study. Ten fetuses would undergo CDH creation, five fetuses would receive fAD treatment and two fetuses would be included as a healthy control group.

CDH

The group undergoing the creation of an iatrogenic diaphragmatic hernia had fetal surgery planned at day 75 of gestation. The ewes were fastened for 16 hours before surgery. Sedation was performed with an intramuscular injection of xylazine chloride (0.2 mg per kg body weight). Anesthesia was induced by intravenous application of thiopental (1 g) and meloxicam (0.25 g per 10 kg body weight). Maintenance of anesthesia was performed with isoflurane (2%) with NO_2 (0.3 l/min) and O_2 (1.6 l/min). 1 g cefazolin was implemented as intravenous antibiotic prophylaxis.

The position of the fetus in the uterus was examined by ultrasound. The abdomen was opened via a midline laparotomy, the uterus luxated out of the abdominal cavity and opened with monopolar cautery. Amniotic fluid was collected, and the fetus carefully extracted and inspected. A fetal thoracotomy was performed in the third lower intercostal space and the thorax opened by bipolar cautery. The diaphragm was exposed by retracting the lung and a 1 cm wide incision was cut at its apex. Omenta or small intestine were pulled through this defect as placeholders (see Figure 3). The

fetal thoracotomy was closed with nonabsorbable sutures and the fetus repositioned in the uterus. Umbilical cord pulsation was carefully manually controlled after replacing the fetus. Amniotic fluid was reinserted, and 400 mg of amoxicillin was given as intra-amniotic prophylaxis. The uterine wall was closed together with the amniotic membranes with a running suture (3/0 Vicryl) and repositioned into the abdomen. A vitality check of the fetus was performed postoperatively by ultrasound. The ewe was allowed to recover after closing of the abdomen and evaluated through the first five postoperative days for any signs of infection.

Abdominal decompression

Fetoscopic abdominal decompression (fAD) was timed for gestational day 95. Sonography confirmed the vitality and position of the fetus inside the uterus as well as the estimated amount of amniotic fluid and the best location to insert the first port. A 24G spinal tap needle (Braun, Germany) was inserted under sonographic guidance and connected to an infusion system containing 0.9% sodium chloride with a temperature of 38° Celsius. Amnioinfusion was performed, applying two to three liters of fluid under sonographic control of the fetus. After sufficient enlargement of the amniotic cavity, the needle was extracted and a 5 mm cutting port (Karl Storz, Germany) was inserted percutaneously under sonographic guidance. The amnioinfusion was connected to the port and a 5 mm videoscope (Karl Storz, Germany) inserted. The position of the fetus was assessed. Two 3 mm ports (Karl Storz, Germany) were inserted under fetoscopic guidance at the position of best triangulation to the left lower abdominal quadrant of the fetus. The fetus was then grasped, and an eight to 13 millimeter long vertical defect was created just lower left to the umbilicus with a hook cautery (Karl Storz, Germany). The layers of the abdominal wall were dissected, and the fetal abdominal cavity opened (Figure 4) and inspected with fetal laparoscopy. After the procedure, amnioinfusion was discontinued and the fluid drained through the ports to the preoperative estimated amount. The ports were removed, the fascia closed with a 1/0 Vicryl u-stitch and the skin closed with 3/0 Vicryl interrupted sutures.

Cesarean section and evaluation

Fetal harvest was performed around day 135. A midline incision was created from the umbilicus downward to the pubic line. The abdomen was opened, and the protruding

uterus palpated to locate the position of the fetus. The uterus was opened with monopolar cautery above the pelvis of the fetus, securing its cut line with clamps for bleeding control. The fetus was extracted and inspected. Directly after ligating and cutting the umbilical line the fetus was euthanized by applying an intracardiac injection with 20 ml of 7.45% potassium chloride. The fetus was then weighed, measured and inspected. The abdomen and thorax were opened and the amount of protruded intestine quantified (Figures 5 and 6). Fetal lungs were collected for further analysis and the trachea sealed to prevent deflation. The placenta was left inside the uterus and the uterine wall was closed without the amniotic membrane with a running suture (3/0 Vicryl) and repositioned into the abdomen. The fascia was closed using a running 0 PDS loop and the skin with interrupted stitches (3-0 Vicryl, all Ethicon, Germany). The ewe was allowed to recover.

Lung stereology

For an objective description of the hypothesized induced lung hypoplasia, lung stereology, a geometric mathematical method, was conducted to offer deeper insight into possible histologically detectable changes. This method is used to offer a qualitative description of possible parenchymal lung changes (Lipsett J et al. 2000). “The methods are based on rigorous sampling of location and orientation, the application of appropriate test systems, and the controlling of the precision of the estimates” (Mühlfeld et al. 2013).

The fetal lungs were fixated for further tissue analysis. A silicon tube was placed into the trachea, fixated, and a solution containing 4% paraformaldehyde, 0.1% glutaraldehyde in 0.2 M HEPES buffer was infused at a constant pressure of 25 cm H_2O over a period of 60 min. Following fixation, the lungs were assigned to systematic uniform random sampling. The randomly selected samples were subsequently fixed with a solution containing osmium tetroxide. The hardened sample was then stained with uranyl acetate and then dehydrated in a solution series of acetone and finally embedded in Technovit 7100 (glycol methacrylate). For microscopic use, three to four samples were taken randomly from each lung and cut to a thickness of 1.5 µm using a rotational microtome.

The prepared samples were placed on microscope slides and stained with toluidine blue. For microscopic observations a light microscope (Olympus BX51) was used which was equipped with an Olympus DP 72 digital camera. The microscope was connected to the computer and the software newCAST (Computer Assisted Stereological Tool, Visiopharm, Horsholm, Denmark) was used for stereological analysis.

To gain a deepened understanding concerning possible histological changes the relative amount of functional parenchyma in relation to the amount of nonfunctional parenchyma was calculated. The calculated ratio gives first insights into structural changes affecting physiological lung function (Mühlfeld et al. 2012). Volume of parenchyma (V(par,lung)) and volume of non-parenchyma (V(nonpar,lung)) were estimated at a magnification of x10. The amount of volume in functional alveoli (V(al, lung)), the mean thickness of intrapulmonary septa and the mean amount of absolute alveolar septa (V(sept, lung) and t(sept), respectively) were calculated at a lens magnifications of x40. The selection of probe slices for microscopy was undertaken by systematic uniform random sampling.

A template consisting of equally distributed points and a template of line grids were placed over the testing field to count the interaction between the test probe (points hitting specific structures, lines intersecting with septal surface etc.); see Figures 7 and 8. The gathered raw data was used to calculate the volume density (volume of a structure per unit of reference volume) and the surface density (surface area per unit of reference volume) according to basic stereological formulas described by Lipsett et al. (2000) and Mühlfeld et al. (2013). Absolute volumes and surface areas were calculated by multiplying the reference volume by the densities and the surface area. Mean thickness of alveolar septa was derived by double division of the total surface area. Due to the small size, statistical testing for significance was not performed.

Results

At the end of the study 11 fetuses were included.

CDH

Mortality after open surgical creation of the CDH was two out of 11 (18%) with a technical success rate of ten out of 11 (91%). In one fetus the CDH closed spontaneously. One fetus died of an accidental splenic laceration intraoperatively.

Abdominal decompression

Fetoscopic abdominal decompression was planned for five fetuses which had undergone successful CDH creation. Access to the pre-operated uterus with the fetoscope turned out to be difficult. Chorioamniotic separation (CAS) and severe oligohydramnios hindered amniotic access. FAD was not possible in two cases due to severe oligohydramnios. During fetoscopic abdominal decompression no intestinal loop was encountered inside of the abdomen in fetuses with CDH. The fetoscopic-laparoscopic abdominal decompression could be performed through the left lower quadrant without any major intraoperative complication according to a previously published protocol by our group for the creation of gastroschisis in fetal sheep (Figure 4) (Bergholz et al. 2012).

Three fetuses were lost after abdominal decompression. One fetus died on the fifth postoperative day, probably due to suture dehiscence. The other two died on postoperative day 25 for unknown reasons. The procedural linked mortality was three out of seven (43%).

Cesarean section and evaluation

Control animals without AD presented an empty abdomen and a thoracic cavity filled with small and large intestine (Figure 5). The effect of fAD resulted in major parts of intestine to herniate into the thorax (Figure 6). Upon evaluation the defect had closed spontaneously in two fetuses which had undergone fAD, resulting in an overall success rate of two out of seven (29%).

The fetal average weight was 3,681 g for the healthy group, 3,400 g for the CDH group and 3,744 g for the CDH and fAD fetuses. The bilateral lung weight was 137.1 g, 68.7

g and 90.7 g, respectively. The lung to fetal weight ratio was 3.71% in the healthy group, 2.02% in the CDH group and 2.39% in the CDH and fAD group (see Table 1).

Lung stereology

Healthy controls showed the highest values for the alveolar volume, except for the contralateral lung (see Tables 2 and 3). The alveolar volume was higher in the CDH-AD+ animal compared to healthy controls. Healthy controls also exhibited a higher amount of alveolar septal volume compared to CDH fetuses.

Alveolar septal thickness increased in CDH animals compared to healthy controls, in which septal thickness decreased after fAD. Total alveolar surface area was decreased in CDH fetuses compared to healthy controls. FAD fetuses displayed a higher total alveolar surface area compared to untreated controls (CDH fetuses).

The data is indicative that a diaphragmatic defect leads to a reduction in bilateral volume of lung parenchyma in an ovine model. Volume of alveolar air space and surface area of alveolar septa in the CDH group improved after fetoscopic abdominal decompression when compared to healthy fetuses. The volume of alveolar septa appears to decrease in CDH without any improvement after fetoscopic abdominal decompression, whereas the mean thickness of alveolar septa increases with CDH and decreases following abdominal decompression.

Discussion

CDH and treatment strategies

To date, the standard treatment of CDH consists of neonatal therapy and pediatric surgery. Using modern ventilation regimes and assistance of ECMO, overall survival has increased in the past decade (Snoek et al. 2016). However, in cases of severe to extreme CDH, mortality still remains very high (Deprest et al. 2011). As such, the use of prenatal interventions has emerged with promising results. Especially the use of FETO is currently the best researched and most advanced approach for prenatal surgical intervention in cases of CDH (Dekoninck P et al. 2011, Montalva et al. 2019). The major disadvantage of FETO is the fact that it alleviates symptoms without correcting the actual defect, the CDH. Even though intrapulmonary pressure increases total lung volume, FETO does not alleviate the high intrathoracic pressure, which is caused by the prolapsed intestinal organs of the CDH (Harrison et al. 1980). For this reason, it is hypothesized that the most effective treatment strategies are those which target the reduction of intrathoracic pressure (Bargy et al. 2006).

Experimental approaches have emerged which consist of reducing the high intrathoracic pressure by inducing an abdominal decompression, as in Porreco's 1994 case study. The advantage of such a method could be a significant reduction of overall intra-abdominal and thoracic pressure, with consequentially normalized lung development. This in turn could greatly improve the survival rates of severe CDH, and especially life quality (Deprest J and De Coppi P 2012). First trials in rabbit and sheep models suggest that iatrogenic gastroschisis improves the quality of lung development in CDH (Chen et al. 2009, Chun et al. 2007, Chun et al. 2004).

When evaluating the work by Chun et al. or Chen et al. there are various points to consider regarding their experimental design. First of all, using a rabbit model to simulate surgical procedures remains problematic. Especially the prominent differences in pregnancy length and uterus anatomy raise the question of transference to humans. Further problems with these experiments are that gastroschisis was established through open fetal surgery with a high mortality of up to 75%. Transference of such a method to human trials would, in respect of the high mortality, have too many

risks for the mother and fetus. It is therefore important to investigate in an animal model how minimally invasive techniques such as fetoscopy could offer alternatives with better survival rates. Experiments by Bergholz et al. have shown that the creation of gastroschisis via fetoscopy is possible and has no major complications, with a low mortality in a sheep model.

Causes and consequences of CDH

A heavily debated question on the topic of CDH is whether lung hypoplasia is due to the herniated intestine, or if it occurs as an independent event before the development of the hernia. Authors such as Ackermann et al. (2005) have investigated mice, which were exposed to nitrogen. They found a mutation on the FOG2 gene and concluded that lung hypoplasia occurs independently from CDH. Others argue that the pleuroperitoneal canal, which according to classic embryological theory is believed to cause the prolapse, is simply too small in the tenth gestational week for the bowel to herniate through (Iritani et al. 1984). Iritani believes that lung hypoplasia is caused by a defect in the diaphragm due to an improper development of the post-hepatic mesenchymal plate. Yet other studies conducted by Kluth et al. (1993) and Bargy et al. (2006) observed that pulmonary hypoplasia is a specific result of liver herniation, compressing the lungs. The observations were verified in humans (Deprest et al. 2011), and it was concluded that the lungs did not show any signs of hypoplasia before the 26th week of pregnancy. Harrison et al. have further shown that in vivo decompression of the thorax correlates with better lung parameters.

Open vs minimally invasive

Even if lung hypoplasia is not an independent event, the above-mentioned studies have shown that a continued compression of lungs by intestine correlates with an increased overall severity of lung hypoplasia. As such, any approach aiming at alleviating the intrathoracic pressure could prove to be beneficial (Harrison et al. 1997).

This study was based on initial findings published by Bergholz et al. in their study “Abdominal wall incision with or without exteriorization of bowel: results from a fetal lamb model for the embryogenesis of gastroschisis” in 2013, in which the authors demonstrated that the creation of a fetoscopically created gastroschisis-like defect is technically possible with a reported mortality of 12.5%. This poses the question why

the mortality increased so dramatically in this study to a high 43%. A major explanation could be the fact that previous studies performed fAD on an unaffected fetus which had experienced no previous surgical interventions (Krebs et al. 2009). In contrast, our study performed fAD on a fetus which had already undergone open fetal surgery, hence greatly increasing the risk of complications (Adzick et al. 2015). Furthermore, a surgically non-naïve uterus is linked to an increased rate of premature rupture of membranes, increased rate of infection and increased probability of fetal death (Lipsett et al. 2000).

Problems and weaknesses of the study

A retrospective analysis revealed an infection in some of our sheep with Chlamydophila abortus or the Schmallenberg orthobunyavirus, a virus which can cause fetal loss and congenital abnormalities of the central nervous systems in ruminants (Peperkamp et al. 2015). Our ewes were tested for infectious pathogens before entering the study, but Schmallenberg orthobunyavirus was not tested for, as its prevalence was considered to be very low in Europe (Beer et al. 2013). The infected animals were excluded from the study. Therefore, it is expected that the mortality will decrease with full development of the surgical technique and chlamydophila-free herds. Nevertheless, fetal demise could also be a direct consequence of fetoscopic intervention.

The most difficult technical aspect was access to the fetus for fetoscopy. Oligohydramnios and chorioamniotic membrane separation (CMS), as mentioned before, obstructed fetoscopic access and manipulation greatly, resulting in increased operating time with subsequentially increased risk of operative complications (Hicks et al. 2017). These obstacles will not be found that pronounced in human cases, as CAS is less often found in the human uterus and fetoscopic abdominal decompression would be performed on a surgically naïve uterus (Kohl T et al. 2009).

During fetoscopic abdominal decompression no intestinal loop was encountered through the opened abdominal wall in CDH fetuses. As experienced in the previous study by Bergholz et al. (2013), omenta and intestine start protruding to the outside of the abdomen once its wall has been opened, following the path of least resistance as hypothesized by Montgomery et al. (1995). The empty fetal abdomen was seen as early as two weeks after creation of the CDH, demonstrating the rapid displacement of

the intestine into the thoracic cavity and rendering it less resistant than the fetal abdominal cavity.

Stereological lung analysis

The stereological analysis and the resulting data favor our hypothesis. An increase in intrathoracic pressure impairs lung development and results in decreased lung parameters (such as decreased alveolar volume, total alveolar surface area and increased thickness of lung septa) as demonstrated in this study and in morphometric analyses by Harrison (1997) and Lipsett (2000). We were able to illustrate that fetoscopic abdominal decompression had a direct effect on lung parameters. Especially the septal thickness decreased after abdominal decompression. The functional gas-exchange area also improved in treated fetuses, represented by the higher total alveolar surface area (Tables 2 and 3). Furthermore, alveolar volume increased in fAD treated fetuses compared to CDH fetuses, indicative of a larger amount of functional parenchyma. Due to the small number of analyzed fetuses a statistical test for significance could not be performed.

In order to widen the stereological analysis, further studies should include the analysis of pulmonary arteries, in order to investigate the effect of fAD and CDH on vessel maturation. It would be of interested to investigate if our model is able to demonstrate an effect on pulmonary vessel maturation indicative of later possible pulmonary hypertension (Mühlfeld et al. 2015), pulmonary hypertension being the most important determinant of neonatal survival (Montalva L et al. 2019).

Summary

This pilot study contains several major obstacles which may hinder the establishment of fetoscopic abdominal decompression altogether. The high mortality of fAD of 43% (3/7) appears unacceptable. Yet, it is important to keep in mind that Montgomery et al. reported a mortality of 75% and higher. Chen et al. described a mortality of 58% in their fetal rabbit model, similar to Chun et al., who describe a mortality of 34%. As such, the high mortality rates in this experiment appear to be concordant with previously published findings, a fact questioning the overall benefit and safety of abdominal decompression for CDH.

Conclusion

This study demonstrates the general technical feasibility of fetoscopic abdominal decompression in an ovine model of diaphragmatic hernia. Although a pilot study, and technically demanding, the findings, especially the stereological data, support the hypothesis that palliative fetal surgery by abdominal decompression improves survival outcome in fetuses with severe CDH. The fetoscopic technique established in this experiment may help establish an alternative minimally invasive prenatal procedure in the future. In light of the high mortality, and the complications associated with fetoscopic abdominal decompression, it seems unlikely for this approach to replace FETO any time soon. Final results of the TOTAL trial and future development of pharmacological therapies will show if fetoscopic abdominal decompression will have to be discarded altogether (Russo et al. 2019, Dekoninck et al. 2011).

Abstract

BACKGROUND: Severe lung hypoplasia in children affected with congenital diaphragmatic hernia (CDH) is attributed to lung compression by intrathoracically prolapsed intestines. Fetal abdominal decompression (AD) appears to be effective in reducing the pressure by directing the growing intestine away from the lungs into the amniotic cavity. The aim of this study was to morphometrically evaluate affected lungs in an ovine model of CDH after fetoscopic abdominal decompression.

METHODS: CDH was created surgically at mid-gestation in 12 fetuses. Two weeks later, an opening was fetoscopically created in the abdominal wall of seven fetuses. The fetuses were retrieved by cesarean section at the end of gestation and the lung structure quantitatively evaluated by stereology.

RESULTS: Surgical establishment of the CDH was successful in 11 out of 12 fetuses with a mortality of two out of 12 (17%). Seven fetuses with CDH were treated with fetoscopic abdominal decompression. Five fetuses with CDH and three healthy fetuses were taken as controls. Fetoscopic abdominal decompression was successful in two cases out of seven (29%). The procedural linked mortality was three cases out of seven (43%). Morphometric analysis indicated that fetuses with CDH had a lower alveolar volume and thicker alveolar septa compared to healthy controls and those treated with AD showed higher alveolar volume and thinner septa compared to untreated CDH.

CONCLUSIONS: Fetoscopic abdominal decompression is technically feasible but remains a high-risk procedure. In comparison to untreated CDH fetuses, AD helped to preserve a higher alveolar volume and thinner septa. These findings support the hypothesis of an alternative palliative fetal surgery for severe cases of CDH apart from tracheal occlusion.

Zusammenfassung

Hinteregrund: Die angeborene Zwerchfellhernie (CDH), in welcher Darm in den Thorax prolabiert, führt durch mechanische Kompression zu einer lungen hypoplasie der Neugeborenen. Fetoscopische intraabdominalle Dekompression (fAD) scheint den intrathorakalen Druck zu reduzieren. Ziel dieser Arbeit ist es, ein fetales Schafsmodell für die abdominelle Dekompression von Feten mit iatrogener CDH zu etablieren und im Rahmen einer Pilotstudie erste sterologische Daten über die Lungenreifung und Entwicklung zu sammeln.

Methoden: Eine iatrogene CDH wurde in 12 fetalen Schafen durchgeführt. Nach zwei Wochen erfolgte in Sieben Feten die fetoscopische abdominelle Dekompression. Am Ende der Schwangerschaft wurden die Feten mittels Sectio geholt und die Lungen sterologisch analysiert.

Resultate: Die chirurgische technische Erfolgsrate in der Etablierung der CDH betrug 92% mit einer Mortalität von 17%. Sieben Feten wurden einer fAD unterzogen. Fünf Feten mit CDH und drei gesunde Feten wurden als Kontrollgruppen genommen. FAD war zu 29% erfolgreich. Die Mortalität betrug hierbei 43%. Sterologische Analyse deutete an, dass Feten mit CDH ein geringeres alveolar Volumen und dicker alveolar Septen haben im Vergleich zu der gesunden Kontrollgruppe. Feten, welche einer fAD unterzogen wurden, zeigten ein höheres alveolar Volumen und dünnere alveolar Septa im Vergleich zur CDH Gruppe.

Zusammenfassung: Fetoscopische abdominelle Dekompression ist technisch möglich bleibt jedoch ein Hochrisikoeingriff. Im Vergleich zu nicht behandelten CDH Feten, zeigte fAD in der sterologischen Analyse eine Verbesserung des Lungenparenchyms mit geringerer Lungenhypoplasie. Diese Daten liefern Unterstützung für die technische Machbarkeit und den Nutzten diesen experimentellen Ansatzes in der Therapie schwerer CDH.

Appendix

Schematic representation of the experimental stages.

Figure 2

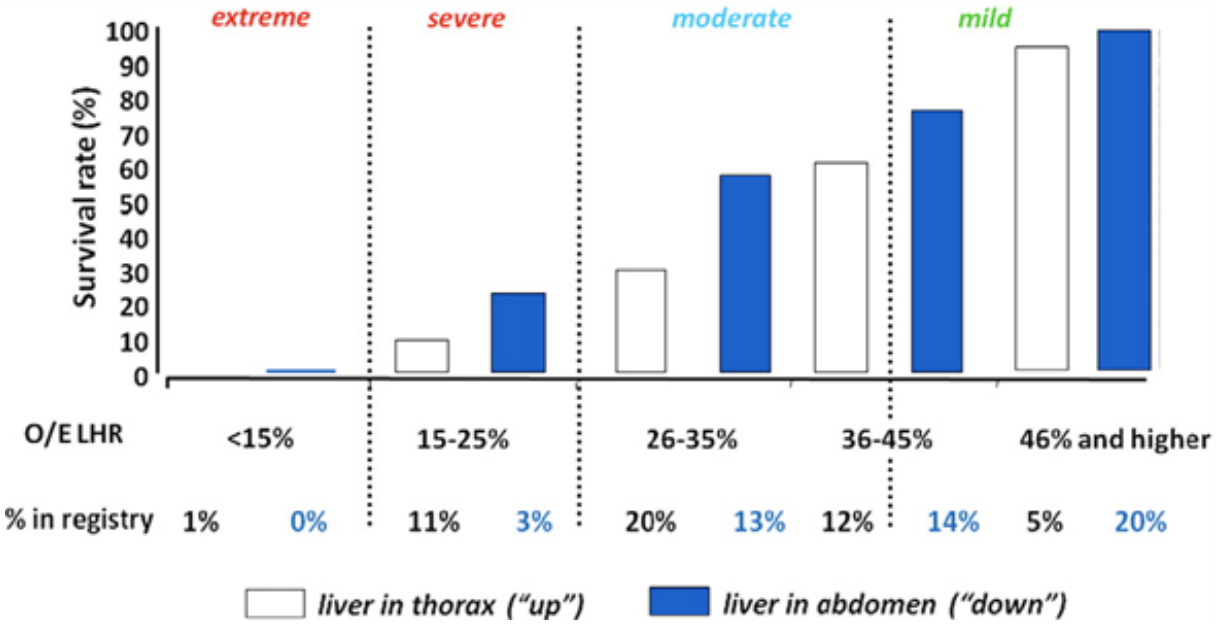
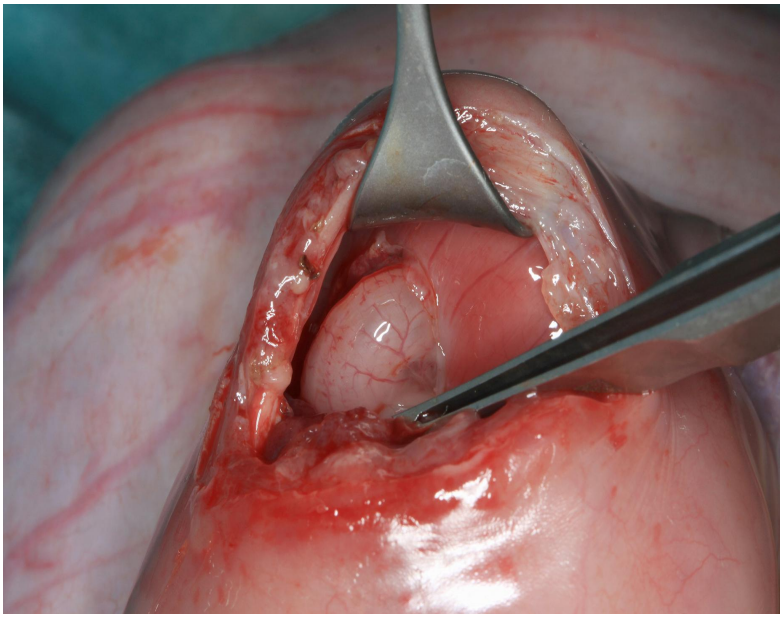


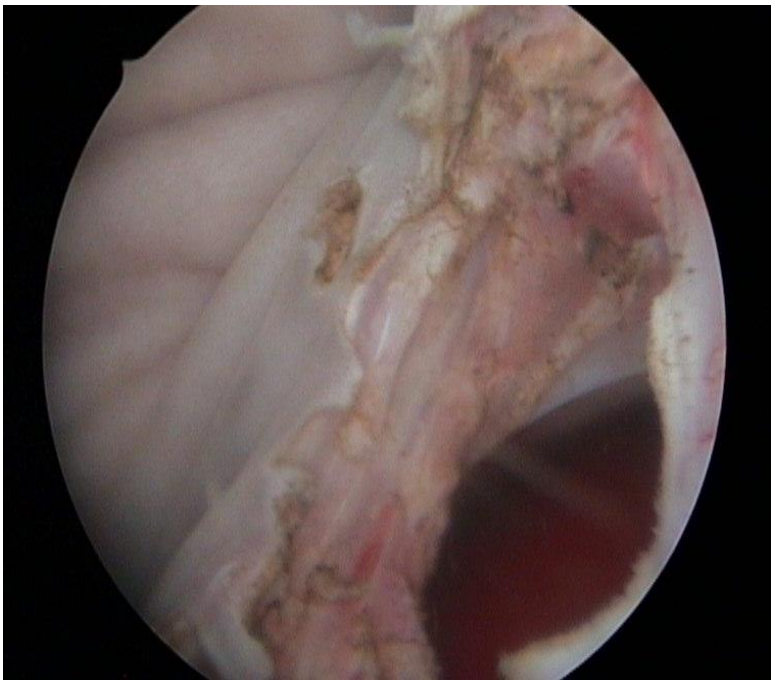
Figure 2 illustrates how applying the O/E LHR is suitable for categorizing the severity of CDH and making a prediction on the survival rate. Figure taken from Deprest J and De Coppi P (2011).

Figure 3



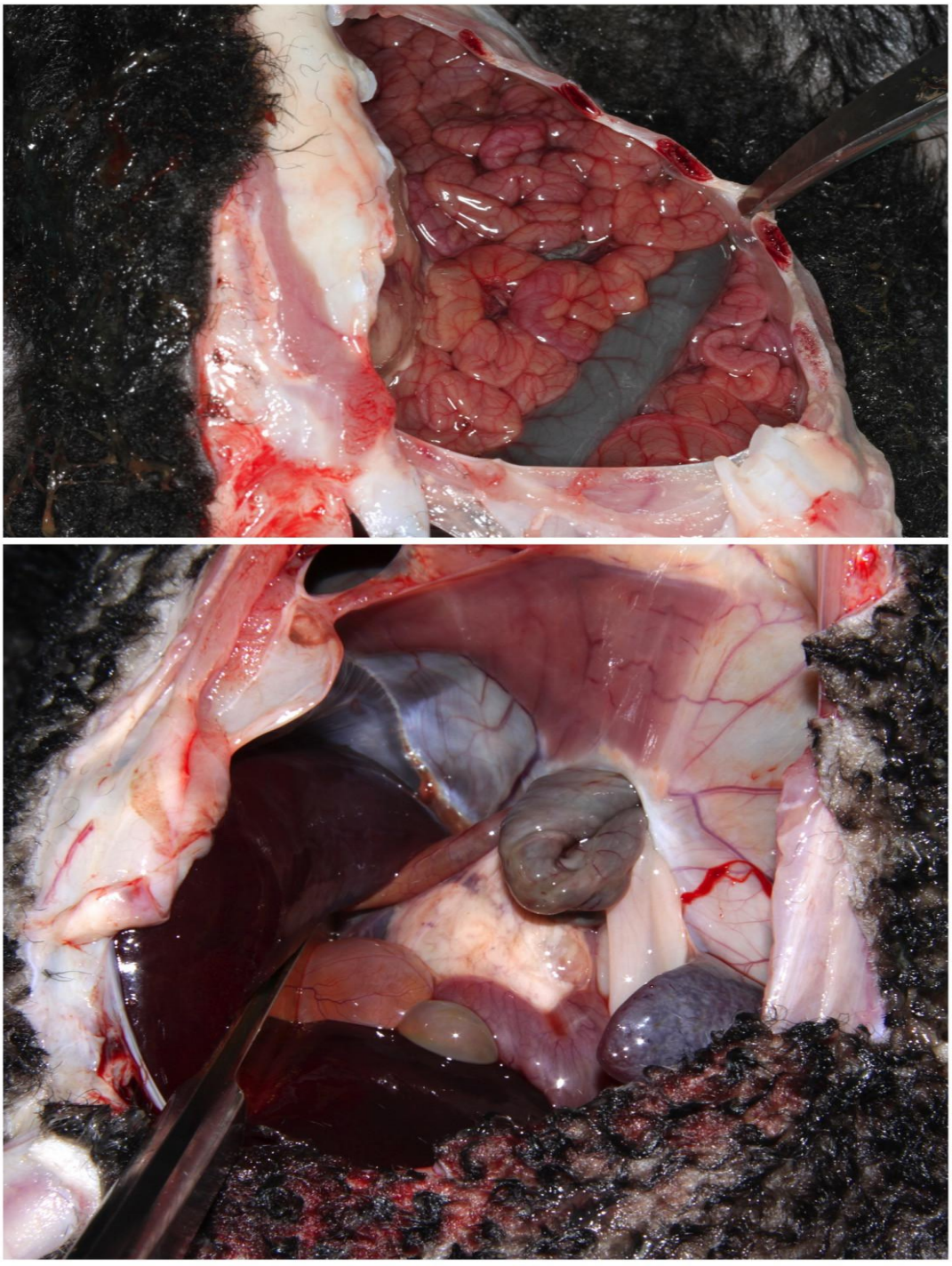
Creation of the CDH. The left thorax is opened by thoracotomy, the view is directed onto the left diaphragm, the ribs are retracted with a hook. A defect has been created in the apex of the diaphragm and an intestinal loop is spontaneously protruding into the thoracic cavity. The lung is held apically by the forceps.

Figure 4



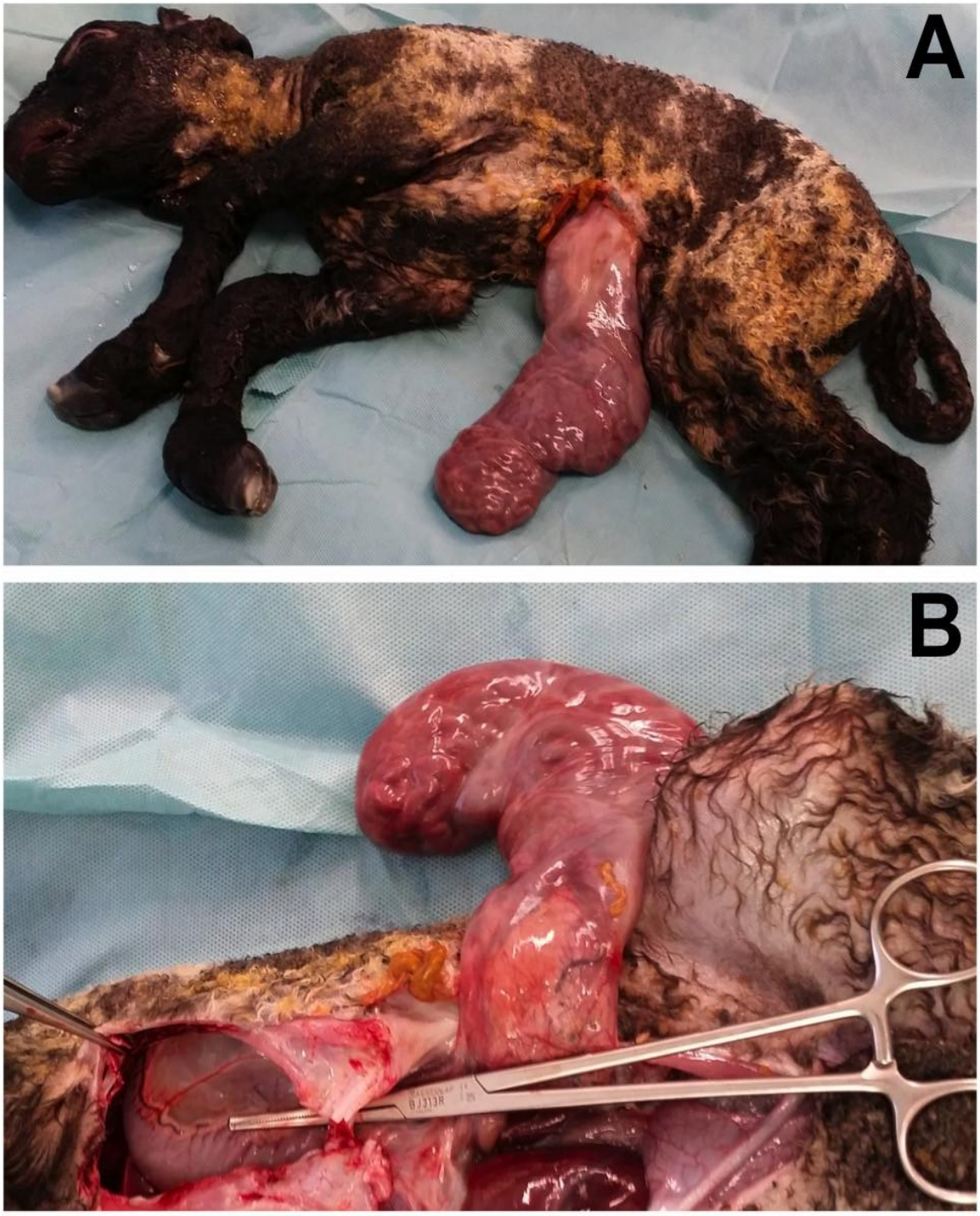
Creation of the AD (fetoscopic abdominal decompression). An incision has been made in the left lower quadrant of the fetus. The abdominal cavity has been opened. No intestinal loop can be found inside of the fetal abdomen. Laparoscopy shows the large fetal liver.

Figure 1



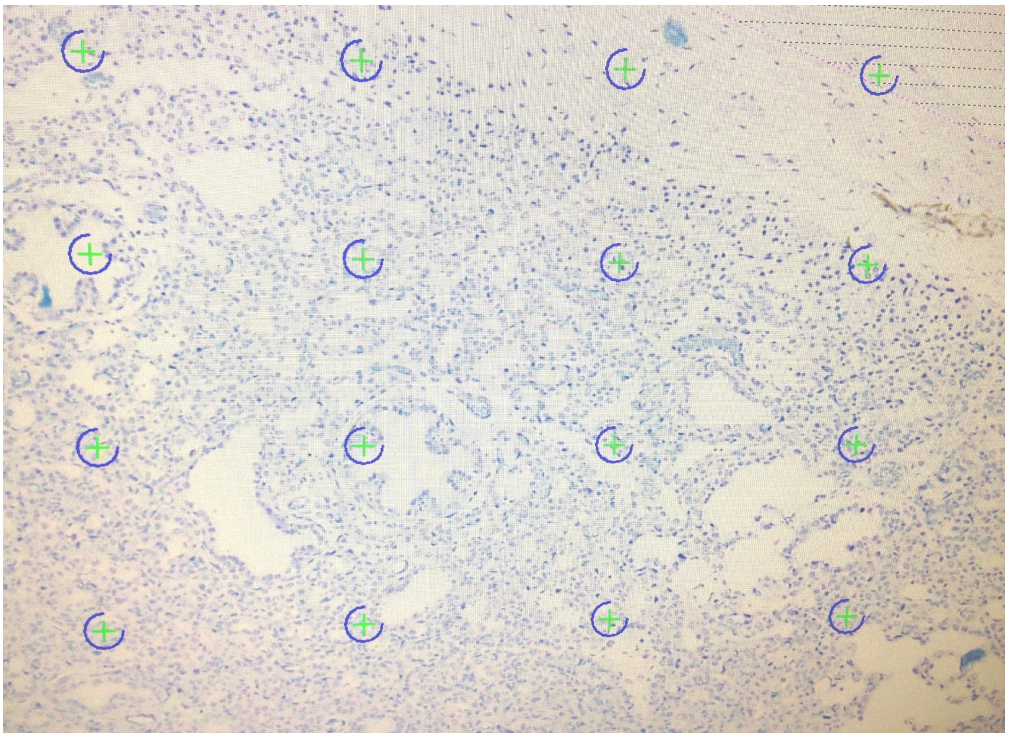
Fetus with CDH. In the thoracic view (A), the lungs cannot be identified due to the overlying small and large intestine. The abdominal view (B) shows the diaphragm with the posterior defect and thoracically herniated small and large intestine.

Figure 2



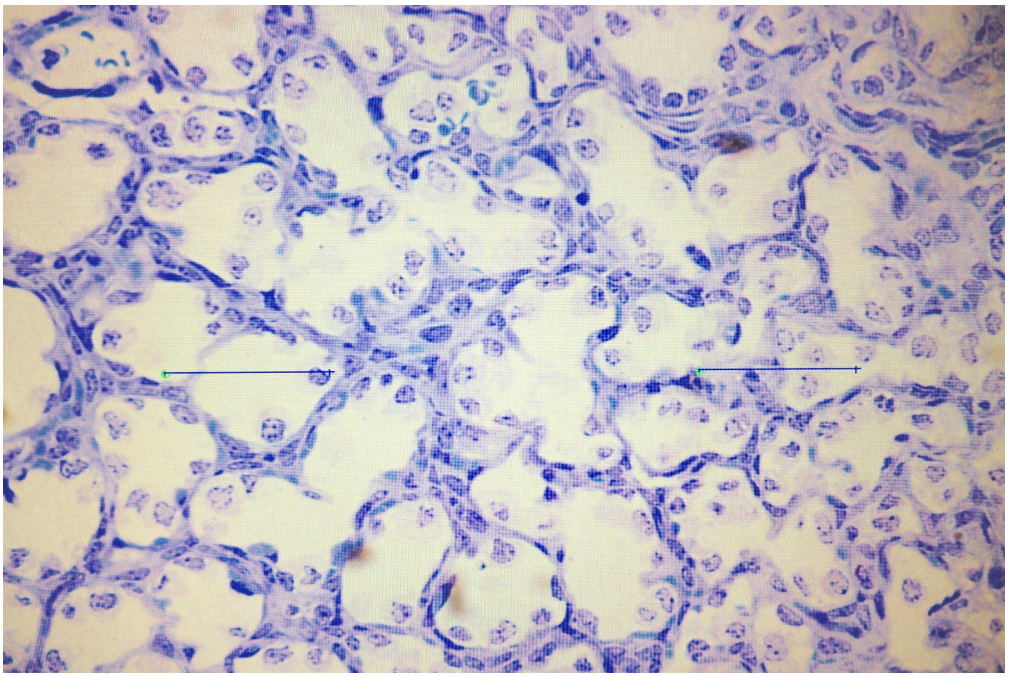
Fetus with CDH after fetoscopic abdominal decompression (CDH+AD+). The intestine can be seen protruding from the fetal abdomen covered with peel (A). The abdominothoracic view (B) displays a posterolateral diaphragmatic hernia (marked by the Overholt clamp) and empty abdomen.

Figure 7



Example of a grid with 5x5 placed dots. The number of dots falling on parenchyma and non-parenchyma were counted.

Figure 8



Example of two lines positioned over the microscope interface.

Table 1

Study groups

Group	n	FBW [g]	FLW [g]	FLW/FBW	Volume [ml]	
					RL	LL
Healthy controls	3	3,819.50	114.50	0.03	77.83	46.46
CDH fetuses	4	4,100.00	56.40	0.01	52.36	27.51
Abdominal decompression in CDH	2	3,744.00	68.80	0.02	79.03	32.43

Volumetric measurements of both lungs. FBW: fetal body weight, FLW: fetal lung weight (R and L), FLW/FBW: ratio; RL: right lung; LL: left lung.

Table 2

Stereological data of the left lung.

LEFT LUNG						
Cohort's left lung	**Lung volume [ml]**	**Volume of parenchyma (ml)**	**Volume of non-parenchyma (ml)**	**Alveolar volume (ml)**	**Septal volume (ml)**	**Septal thickness [µm]**
Healthy						
1	**80.80**	**69,37**	**11,43**	**59,48**	**9,89**	**3,26**
2	**71,20**	**57,47**	**13,73**	**40,13**	**15,70**	**4,74**
3	**61,80**	**51,97**	**9,83**	**36,89**	**13,33**	**4,21**
Average	**71,27**	**59,60**	**11,66**	**45,50**	**12,97**	**4,07**
CDH fetuses						
4	**31,12**	**14,12**	**17,00**	**7,00**	**5,87**	**9,98**
5	**71,19**	**49,08**	**22,11**	**19,63**	**23,47**	**9,96**
6	**50,80**	**35,13**	**15,67**	**21,02**	**12,55**	**7,62**
7	**22,18**	**11,89**	**10,29**	**6,03**	**3,79**	**8,39**
Average	**43,82**	**27,55**	**16,27**	**13,42**	**11,42**	**8,99**
Fetoscopic abdominal decompression						
8	**33,45**	**22,66**	**10,79**	**15,79**	**5,91**	**5,78**
9	**31,40**	**21,97**	**9,43**	**15,79**	**6,18**	**5,82**
Average	**32,43**	**22,32**	**10,11**	**15,79**	**6,04**	**5,80**

Table 3
Stereological data of the right lung

RIGHT LUNG

Cohort's right lung	Lung volume [ml]	Volume of parenchyma (ml)	Volume of non-parenchyma (ml)	Alveolar volume (ml)	Septal volume (ml)	Septal thickness [µm]
Healthy						
1	130.46	106,92	23,54	92,90	14,02	3,05
2	133,20	106,91	26,29	74,46	31,21	5,41
3	103,20	78,07	25,13	57,79	19,52	4,30
Average	122,29	97,30	24,99	75,05	21,58	4,25
CDH fetuses						
4	52,46	29,99	22,47	15,89	11,55	7,74
5	102,56	65,04	37,52	32,52	32,52	8,34
6	62,38	41,27	21,11	23,29	16,27	17,14
7	46,90	23,01	23,89	15,18	6,60	7,15
Average	66,08	39,83	26,25	21,72	16,74	10,09
Fetoscopic abdominal decompression						
8	74,46	63,81	10,65	53,52	10,29	3,63
9	83,60	58,23	25,37	40,55	13,45	3,90
Average	79,03	61,02	18,01	47,03	11,87	3,77

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„Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt“

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Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
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