Maternal hyperoxygenation test in fetuses undergoing FETO for severe isolated congenital diaphragmatic hernia

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This article has been accepted for publication in Ultrasound in Obstetrics & Gynecology and is currently being edited and typeset. Readers should note that this article has been fully refereed, but has not been through the technical editing, copy-editing and proof correction process. Wiley-Blackwell and the International Society of Ultrasound in Obstetrics and Gynecology cannot be held responsible for errors or consequences arising from the use of information contained in this article; nor do the views and opinions expressed necessarily reflect those of Wiley-Blackwell or the International Society of Ultrasound in Obstetrics and Gynecology
**Acknowledgments:** The European Commission (EC) supports this work in its 6th Framework and Marie Curie Fellowship Programme (EuroSTEC; LSHC-CT-2006-037409; MEST CT2005 019707); the Flemish government via its Instituut voor Wetenschap en Technologie (IWT-070715). E.D. J.J., L.G., T.V.M. are recipients of a grant from the EC. J.D. and K.A. are recipients of a “Fundamental Clinical Researcher” grant of the Fonds Wetenschappelijk Onderzoek-Vlaanderen (1801207N and 1800209N).
Abstract

Rationale: Pulmonary hypoplasia and hypertension are the main problems in newborns with isolated congenital diaphragmatic hernia (CDH). The outcome can be prenatally predicted by the measurement of contralateral lung size. Prenatal evaluation of lung vasculature has been much less investigated, and there are no data on the ability to predict pulmonary hypertension.

Objective: To predict neonatal survival and pulmonary hypertension by measurement of fetal pulmonary artery reactivity to maternal hyperoxygenation in fetuses with severe CDH treated by fetoscopic endoluminal tracheal occlusion (FETO).

Methods: 38 fetuses underwent FETO around 28 wks and the balloon was removed at 34 weeks. We performed a hyperoxygenation test and measured the lung-to-head ratio before and after each procedure. Outcome measures were neonatal survival, occurrence of pulmonary hypertension and its response to inhaled-NO (iNO)

Results: Fetuses who survived had a larger increase in lung size and decrease of resistance in the first branch of the main pulmonary artery, than those who died. Both measures were also predictive of pulmonary hypertension unresponsive to iNO. The hyperoxygenation test and lung-to-head-ratio were both best predictive for neonatal survival when measured following balloon removal (p<0.002). Discriminant analysis confirmed that these two parameters are independent predictors of outcome.
**Conclusions:** In fetuses undergoing FETO, pulmonary vascular reactivity in relation to oxygen and lung size are independent predictors of neonatal survival, and pulmonary hypertension. The hyperoxygenation test merits further study in expectantly managed cases.

**Key words:** Congenital Diaphragmatic Hernia, hyperoxygenation test, pulmonary hypertension, lung vascular reactivity.
Introduction:

Congenital diaphragmatic hernia (CDH) is a surgically correctable defect with an incidence of around 1/2,500 births, and with an overall survival rate of 70% when the condition is isolated\(^1\). Prenatal imaging is used to predict outcome, which essentially relies on lung size and liver position\(^5\). The best validated prediction method is based on the measurement of the contralateral lung area and head circumference, corrected for gestational age (Observed/Expected Lung to Head Ratio [O/E LHR])\(^6,7\).

In essence CDH results in markedly diminished alveolar air space, but also a reduced cross-sectional area of the vascular bed. Vessels show adventitial thickening, medial hyperplasia and peripheric extension of the muscle layer into the smaller intra-acinar arterioles\(^8,9,10,11\). These are believed to make CDH-lungs more susceptible to vasoconstrictive stimuli, causing pulmonary hypertension (PHT)\(^12,13\). Many efforts have been made to assess vascular development in the prenatal period, e.g. by measuring number of branches, vessel diameters, flow velocimetry or flow volume\(^14,15,16,17,18,19\). The hyperoxygenation test of pulmonary vascular reactivity is another method to determine the vascular response to increased fetal blood oxygenation.

It is based on the observation that at birth, pulmonary flow increases dramatically coinciding with a dramatic drop in the resistance of the pulmonary circulation\(^20,21\). This response can be mimicked prenatally by administration of supraphysiologic concentrations of oxygen to the mother; In fetuses with normal lung development, this causes a decrease of pulmonary vascular resistance, that can be measured by ultrasound as a difference in pulsatility index (PI) values in
fetal pulmonary artery, before and after oxygen exposure. Test typically turns reactive around 31 to 36 weeks, but there are no true normative curves. Broth et al described its use in fetuses at risk for pulmonary hypoplasia showing that hyperoxygenation test is predictive for neonatal survival.

In view of their poor outcome, we offer prenatal therapy to fetuses with severe, isolated CDH (predicted mortality >80%)\(^{24,25}\). We perform fetal endoscopic tracheal occlusion (FETO) by a balloon, which is inserted at 28 weeks and removed at 34 weeks. Early clinical experience suggested a survival rate exceeding 50% as compared to <15% in same severity cases\(^{27}\). This is an investigational procedure and a trial is currently being undertaken\(^5\). Tracheal occlusion has been shown to increase lung size, as measured by ultrasound and MRI\(^{28,29}\). The clinical effects of FETO on vascularization have so far not received much attention. Herein, we have measured changes in fetal pulmonary arterial reactivity in fetuses with severe CDH undergoing tracheal occlusion or balloon removal. Further we analyzed the predictive value of these observations towards survival and/or the occurrence of PHT.
Materials and methods:

This is an ongoing prospective, longitudinal study carried out in the University Hospital Leuven (Belgium). This report includes observations on 38 fetuses, followed up between 26 and 36 weeks gestational age (GA) from January 2006 to August 2007. All had liver herniation and a O/E LHR<27%, hence a poor predicted outcome, for which reason they underwent FETO. Principal outcome measures were O/E LHR and maternal hyperoxygenation test results. O/E LHR and HPVR were obtained at 5 different time points (T): 24 hours before and after FETO (T1 respectively T2), and 24 hours before and after the removal of the balloon (T3 respectively T4) and whenever possible, a fifth measurement prior to birth (T5). For each measurement the exact gestational age was noted. Ultrasound was performed using a Voluson 730 Expert (GE Medical Systems, Milwaukee, WI, USA) with a RAB4-8L transducer. Doppler settings were standardized as follows: angle of insonation < 10°, low filter level and low spatial peak temporal average power output. Flow velocity waveforms (FVW) during at least three cardiac cycles were obtained from the first branch pulmonary artery (PA), and expressed as pulsatility index (PI). First, the PA of the contralateral lung was located with color Doppler, then its first arising branch to record a FVW close to its origin. Doppler measurements were performed twice: first, with the mother exposed to room air and second, following 10 min of inhalation of a mixture of room air and oxygen (9L/min) by face mask. All recordings were obtained during episodes of fetal inactivity and apnea. Pre- and post-hyperoxygenation PI values were compared and the difference, expressed as a percentage of the pre-hyperoxygenation value (delta PI or ?PI), was calculated.
All fetuses were delivered in tertiary level centers where patients were managed within the limits described in a protocol recently drafted by a European consortium of neonatal treatment centers\textsuperscript{30}.

Postnatal outcome measures were survival or death at discharge from the hospital, the presence or absence of neonatal (<28d) pulmonary hypertension (PHT), with evidence on cardiac ultrasound imaging of predominant unidirectional right to left shunt. Standard care in the occurrence of PHT was the initiation of inhaled nitric oxyde therapy (iNO), with a starting dose of 10-20 ppm, at oxygenation index (MAP x FiO2 x 100/ PaO2) =20 or a pre-postductal saturation difference =10 %.

Refractory PHT was characterized by a lack in response to iNO, defined as drop of 10-20% in the pre-postductal saturation difference or an increase of PaO2 of 10-20 %.

In 30 cases, the in vivo Doppler measurements were performed twice by the same operator (ED) to determine intra-observer variability; as well as by a second operator (JJ) to determine inter-observer variability. Comparisons were made using one way ANOVA with Tukey Kramer HSD test for multiple comparisons, and with two groups for comparison between survivors and non-survivors or those with and without pulmonary hypertension. Logistic regression analysis was used to study the linear relations between the O/E LHR and PI values and neonatal outcome (neonatal survival, severe PHT). Receiver Operating Characteristic (ROC) curves and corresponding areas under the curve (AUC) were obtained at different time points. Discriminant analysis using canonical plot was used to discriminate positive and negative outcomes using the principal
outcome measures. To determine inter and intra-observer variability, matched pairs of Doppler measurements were analysed according to Bland and Altman with correction for multiple testing (Bonferroni) and Fisher Intra Class Correlation. Statistical analysis was performed with JMP Version 7.0 (SAS Institute Inc, Cary NC USA, 2007). P< 0.05 was considered statistically significant. Our FETO programme and this study were approved by the Ethics Committee of the University Hospitals Leuven, including parents written consent.
Results:

Inter- and intra-observer variability of PI measurement.

Bland–Altman analysis of PI measurements as obtained by two sonographers, showed a mean difference or bias of 0.027 (95% confidence interval: 0.102 - 0.157), and a precision (standard deviation of the difference) of 0.06 (t ratio=0.44 ;p= 0.66). Paired measurements done by the same sonographer showed a Fisher Intra Class Coefficient of 0.8

Relevant observations are displayed in Table 1. Summary characteristics of the entire group were a mean O/E LHR of 20.1 % (range: 8.5-27.1 %; severe lung hypoplasia) prior to balloon insertion, a mean GA at FETO of 28.3 (26.4-32.3) weeks and a mean GA at delivery of 37.2 (32.3-41.0) weeks. In 6 patients (16%), the balloon was removed prior to 34 weeks because of ruptured membranes and/or preterm contractions. They eventually also delivered prior to 34 weeks, which precluded an additional measurements after balloon removal. Mean GA at removal of the balloon in the other patients was 34.1 (31.1-35.3; n=32) weeks. Mean O/E LHR prior to removal (T3) was 40.2 % (range: 11.3-100.9) and thereafter 32.3 % (range15.3-81.0). The mean interval between removal and delivery was 3.6 wks (3 d-6.1 wks), which occurred at a mean of 35.6 weeks (range: 28.4-40.2).

In this cohort, early (<7d) resp. late (<28d) neonatal survival was 18 (47%) resp. 16 (42%). Fourteen (37%) babies survived till discharge. They had an O/E LHR=20.4 % resp. 41.5 % at T1 resp. T4. Five of the survivors till discharge did not have PHT, and of the remaining nine, eight responded well to iNO, leaving only one baby with refractory PHT. Conversely, 24 (63%)
newborns died at some stage. The mean O/E LHR at T1 resp. T4 in non-survivors was 19.8 % resp. 25.8%. Whereas the latter is significantly lower than that of survivors (p=0.011), there was no difference in O/E LHR at baseline (T1) between future survivors or neonatal deaths. All non-survivors had PHT at some stage. Twenty-one (86%) had refractory PHT, the remaining 3 responded to iNO therapy.

There were 30 left-sided and 8 right-sided cases. There were no differences in O/E LHR at baseline, neither at T4 for left vs. right cases (20.2 resp. 20.3%). There was a trend but not significantly better survival rate in left cases (12/30 vs. 2/8). Of the 2 surviving right CDH, one had refractory PHT. Of those 6 right CDH that died, 5 had refractory PHT.

Longitudinal data

Evolution of O/E LHR and ?PI over the observation period is graphically displayed for the entire population (fig.1A), as well as broken down for occurrence of refractory PHT (fig. 1 B-C ). In essence, both ?PI and O/E LHR increase between baseline and T3. The O/E LHR rises within 48 hours (T1 to T2), whereas the ?PI requires a longer time period to increase significantly. From T3 onwards, there is again a different course. The O/E LHR drops significantly after balloon removal, whereas this does not happen for ?PI.

Table 2 are the results of a logistic regression analysis for neonatal survival as well as the presence of refractory PHT with ?PI as predictive parameter. Whereas ?PI prior to balloon placement (T1) was not significantly related to neonatal outcome, it did predict survival when measured 24 hours after FETO (T2) (p=0.0073). Measurement of PI at T2 showed a trend for prediction
of the occurrence of refractory PHT (p=0.0593). The differences in PI measurements between future survivors or babies with PHT, persist till T3 (prior to balloon removal) and persist immediately after (T4).

Again, the O/E LHR follows a slightly different course, with a steep change following balloon insertion and removal. At T1, there was a significant difference in O/E LHR between fetuses eventually developing refractory pulmonary hypertension versus those who did not, whereas O/E LHR in eventual survivors and non-survivors was not different. Beyond that time point, the differences in O/E LHR between survivors and non-survivors, or between those to did develop PHT, were all significant.

The above suggests that the two parameters carry different information. Discriminant analysis was used to assess the discriminative value of ?PI and O/E LHR. We did so at T4, in order to obtain the maximal classification into 4 prognostic groups, i.e. survivors and not survivors, and those with refractory PHT and those without. Fisher exact test between the actual outcome and predicted outcome (by discriminant analysis) showed a significant discrimination of survival (p=0.0036) and occurrence of refractory PHT (0.002). The classification by discriminant analysis both for ?PI 4 and O/E LHR is graphically displayed in Figure 2A (survival) resp. 2B (pulmonary hypertension). On these plots the misclassified cases (squares) are indicated as well. In total 7 were misclassified for survival, of which 2 by LHR and 5 by delta PI. There were 5 misclassifications in terms of pulmonary hypertension, of which 2 by LHR and 3 by delta PI. The clinical data of misclassification in terms of survival are detailed in Table 3. To the best of our understanding, we could not determine a specific
pattern for misclassification, except perhaps one case dying from a cause not related to pulmonary hypoplasia (case 6, who died from sepsis).
Discussion:

Today not many studies have attempted to predict the occurrence of unresponsive pulmonary hypertension in the prenatal period. Herein we used the hyperoxygenation test for this purpose, but in a selected population of fetuses with severe CDH. We first demonstrated the reproducibility of the hyperoxygenation test in fetuses with severe CDH. We then assessed contralateral O/E LHR, and performed hyperoxygenation test around the time of tracheal occlusion and its reversal. Both prenatal parameters were used to predict subsequent neonatal survival and pulmonary hypertension. Even in this selected population, within the narrow range of severe CDH, O/E LHR and hyperoxygenation test independently predicted outcome. Both provided different information on lung development at different times along the track of tracheal occlusion and its reversal. Their predictive value improves as pregnancy proceeds, but with a different course for both parameters.

Most prenatal prediction studies have aimed to predict neonatal death, irrespective of its cause. The majority of studies are based on the measurement of lung dimensions, either planimetric or volumetric. The best studied predictive method is the LHR, which in the recent past has been the subject of standardization and normative studies. Also its predictive value following FETO has been documented, both prior to the operation as well as thereafter. Our study confirms the latter observations.

Newborns with CDH are often affected by PHT, which is responsible for neonatal deaths and significant morbidity in a considerable number of cases. PHT is believed to find its anatomical basis in a reduced cross sectional
diameter of the pulmonary vascular bed, as well as increased medial and advential thickness\textsuperscript{9,11,37}. Also, there might be impaired vascular growth, which can directly affect alveolarization and lung growth\textsuperscript{38}. The fetal pulmonary circulation is characterized by a high vascular resistance, which is regulated by numerous factors including endothelins, VEGF and NO\textsuperscript{39,40}. Abnormal molecular mechanisms could be responsible for the pathogenesis of the increased pulmonary vascular reactivity in CDH\textsuperscript{41,42,43,44}.

In our study, O/E LHR predicts the occurrence of PHT. Although this relationship has not been often studied, it should not be a surprise. Intra-uterine airway and vascular development are closely tied\textsuperscript{45,46}. O/E LHR is a proxy of lung size, and given vascular development parallels that of airways, LHR is likely to be an indirect indicator of pulmonary vascular development. Vascular development has been assessed by anatomical measurement of the major pulmonary arteries and nomograms were recently established by Ruano et al.\textsuperscript{47} The group from Necker (Paris) suggested to use 3D power Doppler to measure three different indicators of pulmonary vascularization. These were different in CDH-fetuses as compared to controls. Also measurements were different in fetuses who eventually developed PHT, as compared to those that had no or responsive PHT\textsuperscript{18}.

Also, flow patterns at different levels of the pulmonary branches may be determined, such as the acceleration to ejection time in the main pulmonary arteries\textsuperscript{17}, the pulsatility index (PI), peak early diastolic reversed flow (PEDRF) and peak systolic velocity (PSV) in the proximal arterial branches \textsuperscript{19,48}. 

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None of these actually demonstrates the ability to vasodilate, which is possible by the hyperoxygenation test.

The hyperoxygenation test has the potential to provide “functional” rather than anatomical information. It measures the ability of pulmonary arteries to vasodilate following oxygen administration. The hypothesis is that fetuses who fail to do so, are more likely to be unresponsive after birth, hence at increased risk for neonatal PHT. Earlier studies were small in size (n=29) and had a heterogeneous population at increased risk for pulmonary hypoplasia, including CDH (n=10)\textsuperscript{23}. Limitation of the test is that it relies on a physiologic feature only present late in gestation. For instance, in this study, the O/E LHR but not the hyperoxygenation test was predictive of PHT at baseline. Hyperoxygenation test was only predictive of unresponsive PHT around the time of balloon removal (34 weeks).

This was a longitudinal study so that both indicators could be measured around the time of occlusion as well as reversal. Interestingly both variables took a different course, suggesting they measure different features of lung development. The increase in lung dimensions, as evidenced by O/E LHR, was maximal within 2 days following tracheal occlusion. Much of it may be due to fluid retention. However, the increase persists at a lower pace thereafter, which has been earlier experimentally observed\textsuperscript{49,50}. Following removal of the balloon, entrapped lung fluid is released which logically leads to a drop in O/E LHR. However, it stays above baseline values, suggesting a net increase in lung size\textsuperscript{51}. In future survivors, the net increase in O/E LHR after removal of the
balloon was significantly higher, suggesting that those had developed more lung following TO.

Changes in the lung vascular reactivity to oxygen took another course. Though there was an increase of ?PI between T1 and T2, it was much less than that of the O/E LHR. A significant increase in ?PI from baseline was observed from the third time point onwards. Following balloon removal (T4) both studied parameters even go in different directions. Whether this is an effect of gestational age, or from stimulated lung development by TO, it cannot be concluded from this study since we do not have data on same severity controls. There is however, experimental evidence in fetal rabbits and lambs, that TO decreases peripheric muscularization as compared to unoccluded controls. The predictive value of ?PI increased even further with gestational age, with an AUC at T4 of 0.8. Vascular reactivity was also different between survivors and non-survivors. Future neonates bound to have refractory PHT were less responsive than those eventually surviving without unresponsive PHT (fig.1C).

We are aware of the limitations of our study. First, we studied only fetuses with severe CDH who also underwent FETO. Ideally, we should have included gestational age and severity matched unoccluded fetuses. Since no nomograms for the hyperoxygenation test have been established, matched normal controls would be required as well. This would have allowed us to discriminate the effect of prenatal interventions from the effect of normal lung growth and differentiation. Second, additional measurements assessing pulmonary parenchymal and vascular development would have been interesting. At the onset of the study, these were still investigational, neither of them have proven their
superiority over what today remains best validated (O/E LHR)$^{55,56,57}$. Also, as endpoints we have chosen two binary variables, i.e. survival and refractory PHT. We did so because of their ultimate clinical relevance, but obviously continuous variables reflecting pulmonary function and circulation would be interesting. Also, we could not readily explain misclassifications in all cases. One last limitation is the clinical relevance of information when it only becomes available late in the third trimester. Whereas, in most countries prenatal management options are very limited, the test would still permit for in utero referral to specialized centers, which may have more postnatal treatment modalities, e.g. as ECMO.

In summary, this study showed that both O/E LHR and hyperoxygenation test can be used to predict neonatal survival and the occurrence of PHT. The parameters independently contribute to their prediction. They follow a different course throughout the occlusion and reversal period. This demonstrates that compound measures, which include documenting different aspects of prenatal lung development, might improve prediction of neonatal lung function.
<table>
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<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
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<tr>
<td>Mean gestational age (weeks)</td>
<td>27.8</td>
<td>28.4</td>
<td>33.4</td>
<td>34.1</td>
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<tbody>
<tr>
<td>Survivors</td>
<td>O/E LHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=14 (36.8%)</td>
<td>20.4 %</td>
<td>39.6 %</td>
<td>48.6 %</td>
<td>41.5 %</td>
</tr>
<tr>
<td></td>
<td>±8.7</td>
<td>±11.4</td>
<td>±14.8</td>
<td>±14.6</td>
</tr>
<tr>
<td>Refractory pulmonary hypertension</td>
<td>ΔPI</td>
<td>7.6 %</td>
<td>19.0 %</td>
<td>24.0 %</td>
</tr>
<tr>
<td>N=1 (7%)</td>
<td>±10.8</td>
<td>±10.5</td>
<td>±13.7</td>
<td>±9.8</td>
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<tbody>
<tr>
<td>Non-survivors</td>
<td>O/E LHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=24 (63.2%)</td>
<td>19.8 %</td>
<td>30.6 %</td>
<td>34.9 %</td>
<td>25.8 %</td>
</tr>
<tr>
<td></td>
<td>±4.8</td>
<td>±12.4</td>
<td>±21.9</td>
<td>±9.7</td>
</tr>
<tr>
<td>Refractory pulmonary hypertension</td>
<td>ΔPI</td>
<td>5.1 %</td>
<td>6.1 %</td>
<td>10.8 %</td>
</tr>
<tr>
<td>N=21 (86%)</td>
<td>±4.9</td>
<td>±7.6</td>
<td>±9.3</td>
<td>±9.6</td>
</tr>
</tbody>
</table>

Table 1. Difference in pulsatility index at the first branch of the contralateral pulmonary artery following oxygen administration (ΔPI) and observed over expected lung to head/ratio (O/E LHR) in the study population at different time points (T1-4). Data are presented broken down according to survival (mean ± SD). Survival % is for survival at discharge.
Table 2. Probability and ROC-area under the curve related to neonatal outcome and occurrence of refractory PHT based on ΔPI at T1 to T4 as a predictor.

<table>
<thead>
<tr>
<th></th>
<th>Outcome (dead vs. alive)</th>
<th>refractory PHT</th>
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<tbody>
<tr>
<td></td>
<td>Prob(χ²)</td>
<td>ROC:area under the curve</td>
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<tr>
<td>ΔPI T1</td>
<td>0.4427</td>
<td>0.54</td>
</tr>
<tr>
<td>ΔPI T2</td>
<td>0.0073</td>
<td>0.78</td>
</tr>
<tr>
<td>ΔPI T3</td>
<td>0.0232</td>
<td>0.71</td>
</tr>
<tr>
<td>ΔPI T4</td>
<td>0.0074</td>
<td>0.80</td>
</tr>
<tr>
<td>Case</td>
<td>Misclassification by</td>
<td>Predicted survival</td>
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<tr>
<td>------</td>
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<td>-------------------</td>
</tr>
<tr>
<td>1</td>
<td>O/E LHR</td>
<td>death</td>
</tr>
<tr>
<td>2</td>
<td>O/E LHR</td>
<td>survival</td>
</tr>
<tr>
<td>3</td>
<td>∆PI</td>
<td>death</td>
</tr>
<tr>
<td>4</td>
<td>∆PI</td>
<td>death</td>
</tr>
<tr>
<td>5</td>
<td>∆PI</td>
<td>survival</td>
</tr>
<tr>
<td>6</td>
<td>∆PI</td>
<td>survival</td>
</tr>
<tr>
<td>7</td>
<td>∆PI</td>
<td>survival</td>
</tr>
</tbody>
</table>

**Table 3.** Cases that were misclassified at T4 in terms of survival. Top two rows were misclassified based on their measured O/E LHR, but the occurrence of PHT was correctly predicted. Abbreviations: O/E LHR: obtained/expected LHR, ∆PI: delta PI, PPROM: premature rupture of membranes, PHT: pulmonary hypertension, NND: neonatal death at discharge.
Legend Figures

Figure 1

A: O/E LHR (solid) and ΔPI (dashed) trend in all fetuses during the study period. Lines indicate 50th, 25th and 75th percentiles.

B-C: The box and whiskers plots are the results broken down for occurrence or absence of refractory PHT as a function of measurement of O/E LHR (B) or ΔPI (C). The box indicates the interquartile range and whiskers 5th ad 95th percentiles.

Figure 2.

Discriminant analysis for the prediction of survival (A) and refractory PHT (B) at T4. Discriminant plots for ΔPI and O/E LHR indicating correctly classified cases, either as death ● or alive at discharge ○. Squares are misclassified cases, either ■ death or □ survival. The two larger circles represent 95% confidence regions of the means (+) of each group.
References:


Delta PI

Refractory PHT
No refractory PHT

P = 0.046
P = 0.0024
P = 0.0006

T1
T2
T3
T4