OBSTETRICS

# The randomized Tracheal Occlusion To Accelerate Lung growth (TOTAL)-trials on fetal surgery for congenital diaphragmatic hernia: reanalysis using pooled data

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**BACKGROUND:** Two randomized controlled trials compared the neonatal and infant outcomes after fetoscopic endoluminal tracheal occlusion with expectant prenatal management in fetuses with severe and moderate isolated congenital diaphragmatic hernia, respectively. Fetoscopic endoluminal tracheal occlusion was carried out at $27^{+0}$ to $29^{+6}$ weeks' gestation (referred to as "early") for severe and at $30^{+0}$ to $31^{+6}$ weeks ("late") for moderate hypoplasia. The reported absolute increase in the survival to discharge was 13% (95% confidence interval, −1 to 28; *P*=.059) and 25% (95% confidence interval, 6−46; *P*=.0091) for moderate and severe hypoplasia.

**OBJECTIVE:** Data from the 2 trials were pooled to study the heterogeneity of the treatment effect by observed over expected lung-to-head ratio and explore the effect of gestational age at balloon insertion.

**STUDY DESIGN:** Individual participant data from the 2 trials were reanalyzed. Women were assessed between 2008 and 2020 at 14 experienced fetoscopic endoluminal tracheal occlusion centers and were randomized in a 1:1 ratio to either expectant management or fetoscopic endoluminal tracheal occlusion. All received standardized postnatal management. The combined data involved 287 patients (196 with moderate hypoplasia and 91 with severe hypoplasia). The primary endpoint was survival to discharge from the neonatal intensive care unit. The secondary endpoints were survival to 6 months of age, survival to 6 months without oxygen supplementation, and gestational age at live birth. Penalized regression was used with the following covariates: intervention (fetoscopic endoluminal tracheal occlusion vs expectant), early balloon insertion (yes vs no), observed over expected lung-to-head ratio, liver herniation (yes vs no), and trial (severe vs moderate). The interaction between intervention and the observed over expected lung-to-head ratio was evaluated to study treatment effect heterogeneity.

**RESULTS:** For survival to discharge, the adjusted odds ratio of fetoscopic endoluminal tracheal occlusion was 1.78 (95% confidence interval, 1.05−3.01; *P*=.031). The additional effect of early balloon insertion was highly uncertain (adjusted odds ratio, 1.53; 95% confidence interval, 0.60−3.91; *P*=.370). When combining these 2 effects, the adjusted odds ratio of fetoscopic endoluminal tracheal occlusion with early balloon insertion was 2.73 (95% confidence interval, 1.15−6.49). The results for survival to 6 months and survival to 6 months without oxygen dependence were comparable. The gestational age at delivery was on average 1.7 weeks earlier (95% confidence interval, 1.1−2.3) following fetoscopic endoluminal tracheal occlusion with late insertion and 3.2 weeks earlier (95% confidence interval, 2.3−4.1) following fetoscopic endoluminal tracheal occlusion with early insertion compared with expectant management. There was no evidence that the effect of fetoscopic endoluminal tracheal occlusion depended on the observed over expected lung-to-head ratio for any of the endpoints.

**CONCLUSION:** This analysis suggests that fetoscopic endoluminal tracheal occlusion increases survival for both moderate and severe lung hypoplasia. The difference between the results for the Tracheal Occlusion To Accelerate Lung growth trials, when considered apart, may be because of the difference in the time point of balloon insertion. However, the effect of the time point of balloon insertion could not be robustly assessed because of a small sample size and the confounding effect of disease severity. Fetoscopic endoluminal tracheal occlusion with early balloon insertion in particular strongly increases the risk for preterm delivery.

**Key words:** congenital diaphragmatic hernia, fetal surgery, fetoscopic endoluminal tracheal occlusion, fetoscopy, prenatal diagnosis, preterm premature rupture of the membranes, pulmonary hypoplasia, randomized controlled trial, ultrasound

## Introduction

Congenital diaphragmatic hernia (CDH) is associated with impaired fetal lung development, leading to neonatal respiratory failure and pulmonary hypertension, which can cause neonatal death. In isolated cases, the survival chances can be predicted prenatally by the measurement of the lung size and the presence of intrathoracic herniation of the liver.[1] The lung area contralateral to the defect is measured on a standardized 4-chamber view of the heart and is divided by the head circumference to obtain the *observed* lung-to-head ratio (LHR); the LHR is then expressed as a percentage of what is *expected* in a normal fetus of the same gestational age (GA) to obtain the observed/expected lung-to-head-ratio (o/e LHR).[2] Fetuses with a left-sided CDH and an o/e LHR <25% are considered to have *severe*

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## AJOG at a Glance

### Why was this study conducted?

Two randomized controlled trials on fetoscopic endoluminal tracheal occlusion (FETO) for isolated fetal congenital diaphragmatic hernia reported a statistically significant improvement in survival in severe hypoplasia but not in moderate hypoplasia.

### Key findings

This reanalysis on the basis of pooled data suggests that FETO increases survival and prematurity in both the severity groups.

### What does this add to what is known?

The discrepancy in the results between the 2 trials is more likely because of performing FETO at an earlier gestational age in severe hypoplasia than in moderate hypoplasia; there was no evidence that the effect of FETO depends on the disease severity.

pulmonary hypoplasia, because their survival chances are <25%.[2] When the o/e LHR is 25.0% to 34.9% irrespective of the liver position or 35.0% to 44.9% with intrathoracic liver herniation, the hypoplasia is *moderate*, and the estimated survival chances are around 55%.[2]

Fetoscopic endoluminal tracheal occlusion (FETO) with a balloon can stimulate lung growth and improve neonatal survival.[3] In a large observational study in fetuses with left-sided severe hypoplasia, FETO at a median GA of 27 weeks resulted in a 49% survival, where survival was only 24% in historic controls who did not have fetal therapy.[4] The significant predictors of survival were GA at delivery and lung size at the time of FETO.[4] Therefore, along the spectrum of severe hypoplasia, those with the highest o/e LHR were the most likely to survive.[5] However, FETO also increased the risk for preterm birth, in turn compromising survival chances.[4,6] Because randomized data were lacking, we designed the Tracheal Occlusion to Accelerate Lung growth (TOTAL)-trials, wherein fetuses were randomized to either FETO or expectant prenatal management, both followed by standardized neonatal care.[7,8] In fetuses with *severe* lung hypoplasia, the balloon was inserted at $27^{+0}$ to $29^{+6}$ weeks gestation, this time point being further referred to as "early"[9]. In *moderate* cases, insertion was at $30^{+0}$ to $31^{+6}$ weeks ("late") to reduce the risks for very preterm birth and considering the higher survival rate when comparing with severe hypoplasia.[10] In both trials, balloon removal was scheduled at $34^{+0}$ to $34^{+6}$ weeks or earlier if clinically indicated.[11] In the trial for moderate hypoplasia, survival to discharge from the neonatal unit was 63% (62/98) in the FETO group and 50% (49/98) in the expectant management group ($P$=0.059; risk difference, 13%; 95% confidence interval [CI], −1 to 28; relative risk [RR], 1.27; odds ratio [OR], 1.72).[10] In the trial for severe hypoplasia, survival to discharge was 40% (16/40) in the FETO group and 15% (6/40) in the expectant management group ($P$=.0091; risk difference, 25%; 95% CI, 6−46; RR, 2.67; OR, 3.78).[9] A reductionist interpretation of these results, in terms of statistical significance, suggests that there is evidence of improved survival when the FETO is <25%, but not when FETO is ≥25%. Alternative interpretations may be that first, there is an overall beneficial effect of FETO that decreases with increasing o/e LHR, and second, that the beneficial effect of FETO is primarily dependent on earlier balloon insertion rather than on o/e LHR. The objective of this study is to use the pooled data from the 2 TOTAL trials to examine the heterogeneity of the treatment effect by o/e LHR. Despite the strong confounding of the timing of balloon insertion with o/e LHR, we also aim to gain insight into the effect of GA at balloon insertion.

## Material and Methods

### Study design, setting, and procedures

This is a reanalysis of pooled individual participant data from 2 multicenter trials in which women were randomized in a 1:1 ratio (without stratification factors) to either expectant prenatal management or FETO. The patients were recruited between 2008 and 2020 at 14 experienced FETO centers in 11 countries (Supplemental Table 1). Both trials had a group-sequential design with 5 preplanned interim analyses for superiority. The primary endpoint was survival to discharge from the neonatal unit; in the trial for moderate hypoplasia, survival at 6 months without oxygen dependency was a coprimary endpoint. The trial for moderate hypoplasia was not stopped early, whereas the trial for severe hypoplasia was stopped after the third interim analysis.

The participants in the present study include all 287 randomized patients who did not withdraw consent (196 moderate and 91 severe hypoplasia) (Figure 1). The 91 cases in the severe hypoplasia group comprise of 80 patients included at the third interim analysis and 11 overrunning patients, that is, who were already randomized when the decision to stop the trial was made.

The primary endpoint is survival to discharge from the neonatal intensive care unit (NICU). The secondary endpoints are survival to 6 months of age, survival to 6 months without oxygen dependency, and the GA at live birth.

### Analysis population

We performed an intention-to-treat analysis: patients were analyzed according to the arm to which they were randomized, irrespective of postrandomization events (Figure 1 provides the details). For the secondary endpoint GA at live birth, we only used data from live born babies (n=279).

### Covariates

The key variables were intervention (FETO vs expectant) and o/e LHR as the central prognostic variable which also determined severity of hypoplasia. The additional variables were early balloon

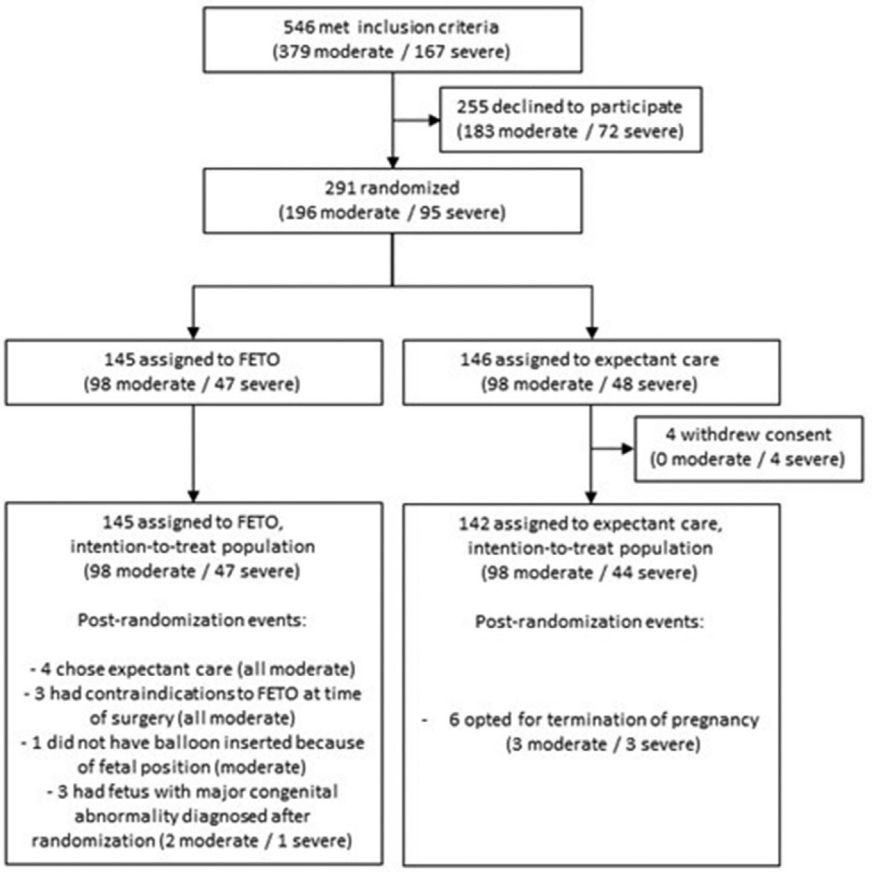
insertion (yes vs no), liver herniation, and the trial to which the participants were recruited (severe vs moderate). The timing of balloon insertion was confounded with o/e LHR, because disease severity determined the fetal surgical protocol. The use of an intervention variable and a timing variable implies the following: the intervention variable estimates the effect of FETO with late balloon insertion and the timing variable assesses the additional effect of early instead of late insertion. The trial covariate aims to capture other sources of heterogeneity between trials apart from the timing of balloon insertion, o/e LHR, and liver herniation. For example, there were differences in the FETO centers (supplemental Table 1) and postnatal care centers. Finally, we evaluated the interaction between intervention and o/e LHR to assess heterogeneity of the effect of FETO (on logit scale).

## Statistical methods and sample size

We used logistic regression (binary endpoints) or ordinary least squares regression (GA at live birth), both with penalized maximum likelihood estimation.[12] For ordinary regression, we examined the model diagnostics and concluded that there was no need to transform the outcome variable. The main reasons for using penalized estimation are as follows: (1) the trial for severe hypoplasia stopped early for superiority, which incurs a risk for optimistic effect sizes,[13,14] (2) the confounding between o/e LHR and the time of balloon insertion and between o/e LHR and liver herniation, (3) the objective of assessing the interaction between intervention and o/e LHR,[15] and (4) the aim to tentatively visualize the predicted outcomes by o/e LHR and liver herniation.

We included all covariates by default without variable selection. We allowed for a nonlinear effect of o/e LHR by using restricted cubic splines with 3 knots.[12] In practice, this added 1 covariate. We evaluated the interaction between intervention and o/e LHR using an alpha of 0.157 (ie, the *Akaike* criterion).[16] However, this does not imply that $P \leq .157$ was used to claim statistical significance. We did not specify an alpha level to declare statistical significance and interpreted results more generally, on the basis of uncertainty and the integration with other evidence and considerations. The statistical analysis was performed using R version 4.0.1 (R core team 2021), using the rms package for regression modeling.[12] Information on sample size and further comments about the analysis are provided in the Appendices A-C.

**FIGURE 1**
**Flow chart combining the 2 randomized TOTAL trials**




*FETO*, fetoscopic endoluminal tracheal occlusion; *TOTAL*, Tracheal Occlusion To Accelerate Lung growth.

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## Results

Table 1 summarizes the baseline characteristics and endpoints (Supplemental Tables 2 and 3 provide trial-specific results). In fetuses randomized to FETO, survival to discharge from the neonatal unit was 54% compared with 39% in those randomized to expectant management. Supplemental Figure 1 displays the GAs at which balloon insertion was done as a function of the o/e LHR at randomization. The adjusted odds ratio (aOR) of FETO with late balloon insertion for survival to discharge was 1.78 (95% CI, 1.05−3.01; $P$=.031) (Table 2). The effect of early vs late balloon insertion was highly uncertain (aOR, 1.53; 95% CI, 0.60−3.91, $P$=.370). When combining these 2 effects, the aOR of FETO with early balloon insertion was 2.73 (95% CI, 1.15−6.49). There was evidence for associations of o/e LHR and liver herniation with survival. Importantly, the interaction between o/e LHR and intervention was weak and it was therefore not added to the model (beta, 0.011; standard error (SE), 0.016;

**TABLE 1**
**Descriptive statistics for baseline characteristics and endpoints in the pooled data**

| Baseline characteristics | FETO (n=145) | Expectant (n=142) |
|---|---|---|
| Maternal age (y) | 31.4 (27.5–34.5) | 30.9 (27.0–34.3) |
| Gestational age (wk) | 28.1 (26.6–29.3) | 27.7 (26.7–29.0) |
| Nulliparous | 70 (48) | 68 (48) |
| Body mass index (kg/m$^2$) | 24.0 (20.9–28.4) | 23.1 (21.1–27.4) |
| Smoking during pregnancy | 11 (8) | 14 (10) |
| Alcohol use during pregnancy | 1 (1) | 1 (1) |
| Ethnicity | | |
| White | 124 (86) | 124 (87) |
| Asian | 9 (6) | 7 (5) |
| Black | 4 (3) | 6 (4) |
| Other | 8 (6) | 5 (4) |
| Ultrasound findings | | |
| Observed/expected lung-to-head ratio (%) | 28.0 (24.0–33.0) | 28.0 (23.2–33.4) |
| Liver herniation | 121 (83) | 116 (82) |
| Deepest vertical pocket of amniotic fluid (mm) | 6.0 (5.0–7.9) | 6.3 (5.7–7.6) |
| Cervical length (mm) | 36 (32–40) | 36 (32–40) |
| Placenta anterior | 83 (57) | 71 (50) |
| Placenta posterior | 56 (39) | 66 (46) |
| Placenta fundal | 6 (4) | 5 (4) |
| Endpoints | | |
| Survival to discharge (primary endpoint) | 79 (54) | 55 (39) |
| Survival to 6 mo of age | 78 (54) | 55 (39) |
| Survival to 6 mo without oxygen supplementation | 63 (43) | 46 (32) |
| Gestational age at live birth (wk) (n=279) | 35.6 (33.8–37.3) | 38.3 (37.0–39.0) |

Data are presented as median (interquartile range) or as number (percentage). There were no missing values for these parameters.

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$P$=.502). The model is visualized in Figure 2. On the basis of this model, Supplemental Figure 2 shows the estimated differences in the probability of survival to discharge for early and late FETO vs expectant management.

The results for survival to 6 months were nearly identical to those of survival to discharge from the NICU (Table 3, Supplemental Figures 3 and 4). Again, the interaction between o/e LHR and intervention was not included (beta, 0.011; SE, 0.016; $P$=.514). For survival to 6 months of age without oxygen supplementation, the results were comparable yet more uncertain (Table 4, Figure 3, Supplemental Figure 5). The aOR of FETO with late balloon insertion for survival to discharge was 1.63 (95% CI, 0.96–2.76). For FETO with early balloon insertion, the aOR was 2.21 (95% CI, 0.83–5.86). The interaction between o/e LHR and intervention was not included (beta, 0.0063; SE, 0.017; $P$=.710). The results for GA at live birth were very different (Table 5, Figure 4). There was evidence of a strong effect of the timing of balloon insertion. The GA at delivery was on average 1.7 weeks earlier (95% CI, 1.1–2.3) following FETO with late insertion and 3.2 weeks earlier (95% CI, 2.3–4.1) following FETO with early insertion compared with expectant management. There was little evidence of an association of o/e LHR and liver herniation with the GA at live birth. Again, the interaction between o/e LHR and intervention was not included (beta, −0.012; SE, 0.023; $P$=.612).

Further analyses (not prespecified) suggested that the effect of FETO on the GA at live birth was partially mediated by preterm premature rupture of membranes (PPROM) (details in Appendix B) and that live birth was delayed by on average 4 (95% CI, 1–7) days per week delay in balloon insertion (Figure 5; details in Appendix C). Nevertheless, a large amount of variation in the GA at live birth was not explained

**TABLE 2**
**Multiple logistic regression analysis for the primary endpoint survival to discharge**

| Covariate | B (SE) | Adjusted OR (95% CI) | *P* value |
|---|---|---|---|
| Intervention: FETO (late insertion) vs expectant | 0.58 (0.27) | 1.78 (1.05–3.01) | .031 |
| Early balloon insertion (yes vs no) | 0.43 (0.48) | 1.53 (0.60–3.91) | .370 |
| Above 2 parameters combined: early FETO vs expectant[a] | 1.01 (0.44) | 2.73 (1.15–6.49) | |
| o/e LHR (%) | 0.076 (0.039) | 1.08 (1.00–1.17) | .0040 |
| o/e LHR, nonlinear | 0.016 (0.042) | 1.02 (0.94–1.10) | |
| Liver herniation (yes vs no) | −0.70 (0.32) | 0.50 (0.27–0.93) | .028 |
| Trial (severe vs moderate) | −0.62 (0.44) | 0.54 (0.23–1.26) | .155 |

*B*, regression coefficient; *CI*, confidence interval; *FETO*, fetoscopic endoluminal tracheal occlusion; *o/e LHR*, observed/expected lung-to-head-ratio; *OR*, odds ratio; *SE*, standard error.

[a] The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

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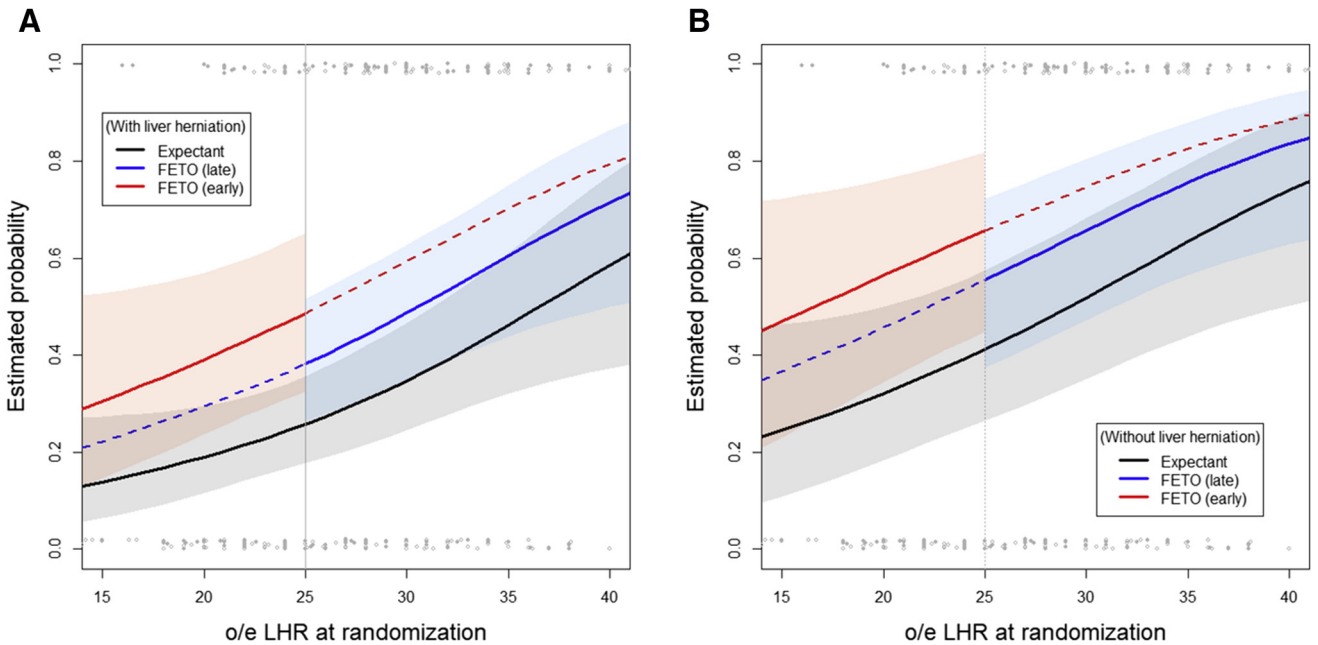
by the moment of insertion (adjusted R-squared, 11%).

## Comment

### Principal findings

There are 4 main findings of this individual patient analysis from the 2 TOTAL trials. First, this study suggests that in both moderate and severe lung hypoplasia, FETO has a beneficial effect on the survival to discharge from the NICU. Second, there is some support for a positive additional effect of early insertion. However, caution is required because of considerable uncertainty in the effects and the strong confounding with disease severity. Third, an adverse consequence of FETO is an increased risk of preterm birth, which is further increased by early balloon insertion. Fourth, there was no evidence that the effect of FETO depends on the o/e LHR for any endpoint. The discrepancy between the results of the 2 TOTAL trials is therefore more likely caused by the difference in the timing of balloon insertion.

**FIGURE 2**
**Estimated probabilities of survival to discharge from the model in Table 2**




Panel **A** refers to cases with liver herniation and panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that are not included in the Tracheal Occlusion To Accelerate Lung growth trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise, 95% confidence intervals are added as shaded areas. The raw patient data are indicated on top (survivors) and at the bottom (nonsurvivors) of the plot. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

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**TABLE 3**
**Multiple logistic regression analysis for the secondary endpoint survival to 6 months**

| Covariate | B (SE) | Adjusted OR (95% CI) | *P* value |
|---|---|---|---|
| Intervention: FETO (late insertion) vs expectant | 0.54 (0.27) | 1.72 (1.02–2.89) | .043 |
| Early balloon insertion (yes vs no) | 0.45 (0.47) | 1.57 (0.62–3.97) | .340 |
| Above 2 parameters combined: early FETO vs expectant[a] | 0.99 (0.44) | 2.69 (1.14–6.35) | |
| o/e LHR (%) | 0.075 (0.038) | 1.08 (1.00–1.16) | .0032 |
| o/e LHR, nonlinear | 0.020 (0.041) | 1.02 (0.94–1.11) | |
| Liver herniation (yes vs no) | −0.72 (0.32) | 0.49 (0.26–0.91) | .024 |
| Trial (severe vs moderate) | −0.60 (0.43) | 0.55 (0.24–1.27) | .162 |

*B*, regression coefficient; *CI*, confidence interval; *FETO*, fetoscopic endoluminal tracheal occlusion; *o/e LHR*, observed/expected lung-to-head-ratio; *OR*, odds ratio; *SE*, standard error.

[a] The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

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## Results in the context of what is known

Extensive animal studies have demonstrated that tracheal occlusion causes fluid retention in the future airways and that this acts as a mechanical stimulus for lung growth (reviewed in Khan[17]). At the molecular level, the DNA synthesis rate is most active 48 h after occlusion and remains stable and elevated at 7 days.[18] The net effect on lung development, however, is the result of a complex interaction between when the occlusion is done, for how long, whether the occlusion is reversed, and whether steroids are used.[17] The current analysis cautiously suggests that an earlier occlusion is of larger benefit. This is in line with animal experiments showing a greater response to experimental tracheal occlusion (TO) in younger than in older fetuses with pulmonary hypoplasia.[19,20] An earlier occlusion combines 2 factors that potentially determine the intensity of the lung response to occlusion, that is, compliance of the chest, which is higher in younger fetuses and hence permits more expansion and a longer duration of occlusion.[21] Earlier vs late occlusion can be simulated in animals that have a longer pregnancy duration, such as sheep. In fetal lambs with CDH, a late TO induced lung growth, but its effect was more prominent when TO was done earlier.[22] Also of interest is the experimental finding that TO is associated with greater growth of the mesenchymal tissue than that of the airways and hence it results in thicker air/blood gas barriers.[21] This may, next to the consequences of prematurity, reduce oxygen uptake across the lungs and could reduce the effect of TO on survival at 6 months without oxygen supplementation.

Also, previous clinical data support the greater effect of early occlusion. In a clinical study on longitudinal changes in lung volume following FETO, the net lung expansion rate, corrected for GA, was on average 1.5% per week after FETO.[23] Remarkably, in that study, GA at FETO was the single independent predictor of the lung volume eventually achieved, and hence, lung size (or the degree of hypoplasia) at baseline was not predictive.[23] On the basis of a model developed on that dataset, late occlusion would result in a net volume increase of 15.2% compared with 35% if the occlusion period started

**TABLE 4**
**Multiple logistic regression analysis for the secondary endpoint survival to 6 months without oxygen dependency**

| Covariate | B (SE) | Adjusted OR (95% CI) | *P* value |
|---|---|---|---|
| Intervention: FETO (late insertion) vs expectant | 0.49 (0.27) | 1.63 (0.96–2.76) | .070 |
| Early balloon insertion (yes vs no) | 0.30 (0.52) | 1.36 (0.49–3.75) | .558 |
| Above 2 parameters combined: early FETO vs expectant[a] | 0.79 (0.50) | 2.21 (0.83–5.86) | |
| o/e LHR (%) | 0.052 (0.040) | 1.05 (0.97–1.14) | .0052 |
| o/e LHR, nonlinear | 0.043 (0.042) | 1.04 (0.96–1.13) | |
| Liver herniation (yes vs no) | −1.16 (0.32) | 0.31 (0.17–0.59) | .0003 |
| Trial (severe vs moderate) | −0.95 (0.46) | 0.38 (0.16–0.96) | .040 |

*B*, regression coefficient; *CI*, confidence interval; *FETO*, fetoscopic endoluminal tracheal occlusion; *o/e LHR*, observed/expected lung-to-head-ratio; *OR*, odds ratio; *SE*, standard error.

[a] The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

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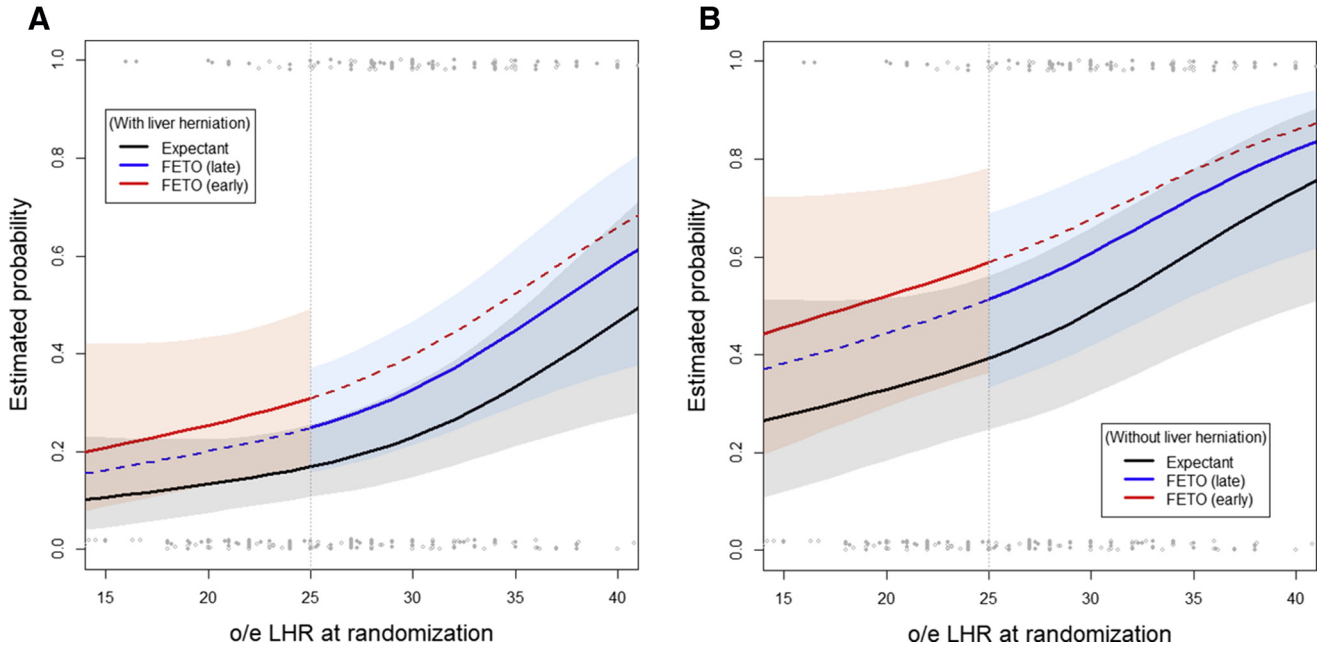
FIGURE 3
**The estimated probabilities of survival to 6 months without oxygen supplementation from the model in Table 4**




Panel **A** refers to cases with liver herniation and panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that are not included in the Tracheal Occlusion To Accelerate Lung growth trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise, 95% confidence intervals are added as shaded areas. The raw patient data are indicated on top (survivors) and at the bottom (nonsurvivors) of the plot. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

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earlier and lasted on average for 6 weeks. These in vivo data and those from an earlier study[24] support the correlation between the duration of occlusion and subsequent lung volume increase. Also in the TOTAL trials, the change in lung size, evidenced by an increase in o/e LHR, was on average +67% over 34 days of occlusion from $27^{+0}$ to $29^{+6}$ weeks onward, compared with only +32% over 24 days of occlusion from $30^{+0}$ to $31^{+6}$ weeks onwards.[9,10]

## Clinical and research implications

In retrospect, our decision to insert the balloon as late as $30^{+0}$ to $31^{+6}$ weeks of gestation in moderate hypoplasia may have resulted in less lung growth, and therefore, inadequate improvement in survival, by comparison with the expectantly managed group. The obvious strategy for extending the duration of TO is to insert the balloon in both the severe and moderate hypoplasia groups at $27^{+0}$ to $29^{+6}$ weeks of gestation and accept the inevitable increase in the risk of prematurity. An alternative strategy is to delay

TABLE 5
**Multiple linear regression analysis for the secondary endpoint gestational age at live birth**

| Covariate | B (SE) | Adjusted OR (95% CI) | *P* value |
|---|---|---|---|
| Intervention: FETO (late insertion) vs expectant | −1.70 (0.31) | −2.30 to −1.09 | <.0001 |
| Early balloon insertion (yes vs no) | −1.51 (0.52) | −2.54 to −0.58 | .0043 |
| Above 2 parameters combined: early FETO vs expectant[a] | −3.21 (0.46) | −4.11 to −2.31 | |
| o/e LHR (%) | −0.0057 (0.046) | −0.096 to 0.085 | .884 |
| o/e LHR, nonlinear | 0.020 (0.049) | −0.077 to 0.12 | |
| Liver herniation (yes vs no) | −0.065 (0.36) | −0.76 to 0.63 | .885 |
| Trial (severe vs moderate) | 0.12 (0.50) | −0.86 to 1.10 | .812 |

*B*, regression coefficient; *CI*, confidence interval; *FETO*, fetoscopic endoluminal tracheal occlusion; *o/e LHR*, observed/expected lung-to-head-ratio; *OR*, odds ratio; *SE*, standard error.

[a] The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

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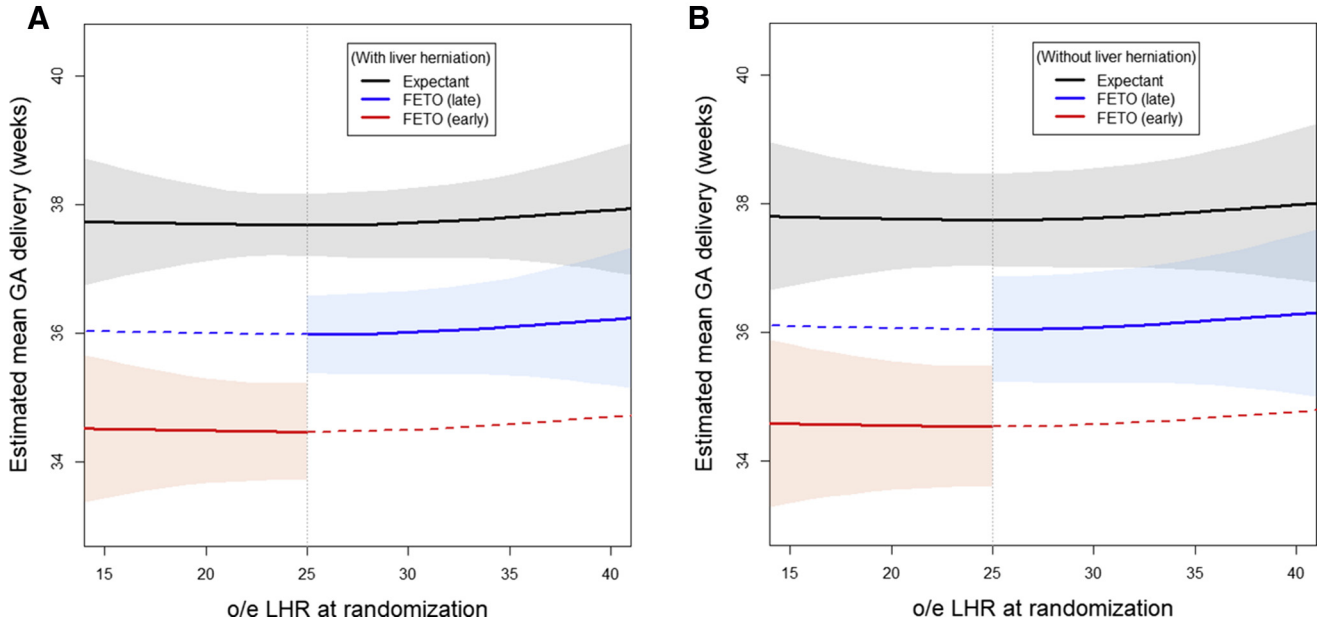
FIGURE 4
**Estimated gestational age at live birth, based on the model from Table 5**




Panel **A** refers to cases with liver herniation and panel **B** to cases with the liver confined to the abdomen. The *dashed lines* indicate situations that are not included in the Tracheal Occlusion To Accelerate Lung growth trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise, 95% confidence intervals are added as shaded areas. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

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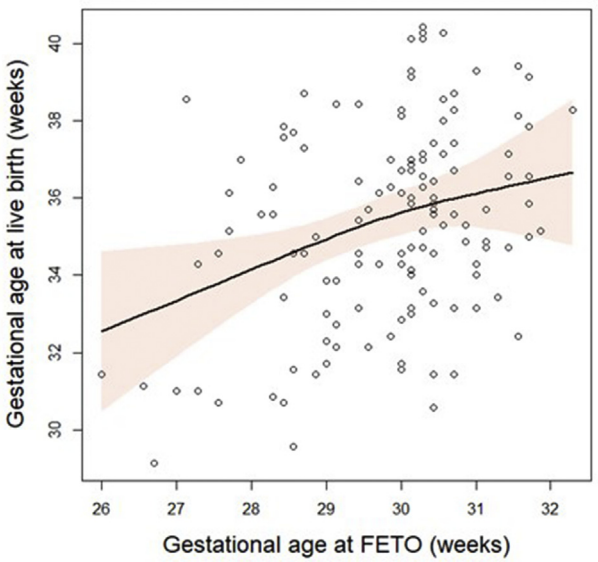
FIGURE 5
**Gestational age at live birth by gestational age at FETO**




A regression line with pointwise 95% confidence intervals is added, see Appendix C for details.

*FETO*, fetoscopic endoluminal tracheal occlusion.

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the removal of the balloon from 34 to 37 weeks. However, the latter would be unwise, because many women will go into labor before the planned day for removal,[10] necessitating emergency removal of the balloon, which is more complex and risky than an elective procedure.[25] In view of these findings, we think that further clinical investigation with an earlier balloon insertion strategy in moderate cases is warranted. Obviously, this should be done with comprehensive documentation including secondary endpoints that cover the potential impact of prematurity while maintaining the same skill set and standards as in the TOTAL trial. Ideally, this would best be addressed by a randomized controlled trial. Given the rarity of the condition, the time it took to complete both TOTAL trials and the controversy on randomizing patients when benefit of fetal surgery is likely may not be evident.[26–32] Prospective high-quality registries that capture consecutive cases and all known

confounding factors could be considered as second best.[31] This should also include factors unrelated to the severity of the condition but to the surgery and postnatal management center, including experience and case load, which may also influence outcome.[33,34] It may be wise to also apply that registration to the rarer forms of CDH, such as right-sided cases.[35]

## Strengths and limitations

Despite the strength of data pooling, this study has limitations. First, the individual trials had limited sample size.[28,36] This leads to uncertain estimates, evidenced by wide confidence intervals. Second, the severe trial was stopped early for superiority. The effect estimates for trials that are stopped early for superiority can be overoptimistic.[13,14] When estimating the overall effect of FETO, the potentially overoptimistic estimation of the effect of FETO in severe cases may be counteracted by the following: (1) the relatively large amount of data from moderate cases, (2) the use of penalized regression, and (3) the lesser known fact that group-sequential trials, when not stopped early, may have pessimistic effect sizes.[37] Early stopping may have affected the interaction between the intervention and o/e LHR toward finding a larger effect of FETO when o/e LHR is low. However, our results provide little support for the existence of this interaction. Third, the FETO balloon was inserted at an earlier GA in patients with severe hypoplasia to maximize lung growth.[23,24] This confounding may artificially increase the FETO effect in severe hypoplasia. We accounted for this in the analysis, yet results should be interpreted with caution. Fourth, recruitment by fetal surgery center was imbalanced and different in the 2 trials. Postnatal care centers were even more numerous, so that the number of neonates per center eventually was very small. We used trial as a covariate to address these and other sources of heterogeneity between trials.

## Conclusions

Our findings suggest that in CDH, FETO increases the survival chances. There was no evidence that these effects depend on the o/e LHR. The results also suggest that earlier occlusion may well improve survival compared with later occlusion, but such a policy would be accompanied by an increased prematurity risk. ■

## Acknowledgments

We thank all the participants and the multidisciplinary teams of all postnatal management centers that make the Tracheal Occlusion To Accelerate Lung growth trials a success.

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The TOTAL trials were registered at www.clinicaltrials.gov, for the severe trial as ClinicalTrials.gov Identifier: NCT01240057, on November 15, 2010, enrolling the first patient on February 11, 2011 and last on February 16, 2020, and for the moderate trial as ClinicalTrials.gov Identifier: NCT00763737, on October 1, 2008, enrolling the first patient on October 10, 2008 and last on May 2, 2019.

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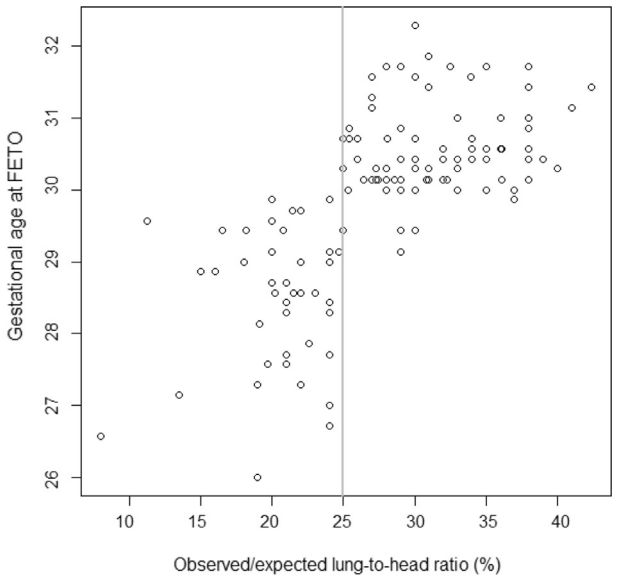
## Supplemental Material

**SUPPLEMENTAL FIGURE 1**
**Gestational age at FETO by observed/expected lung-to-head ratio**




Scatter plot of the observed/expected lung-to-head ratio and gestational age at FETO in patients randomized to FETO and in whom FETO was performed/attempted (n=138). The *vertical line* demarcates the severe and moderate cases.

*FETO*, fetoscopic endoluminal tracheal occlusion.

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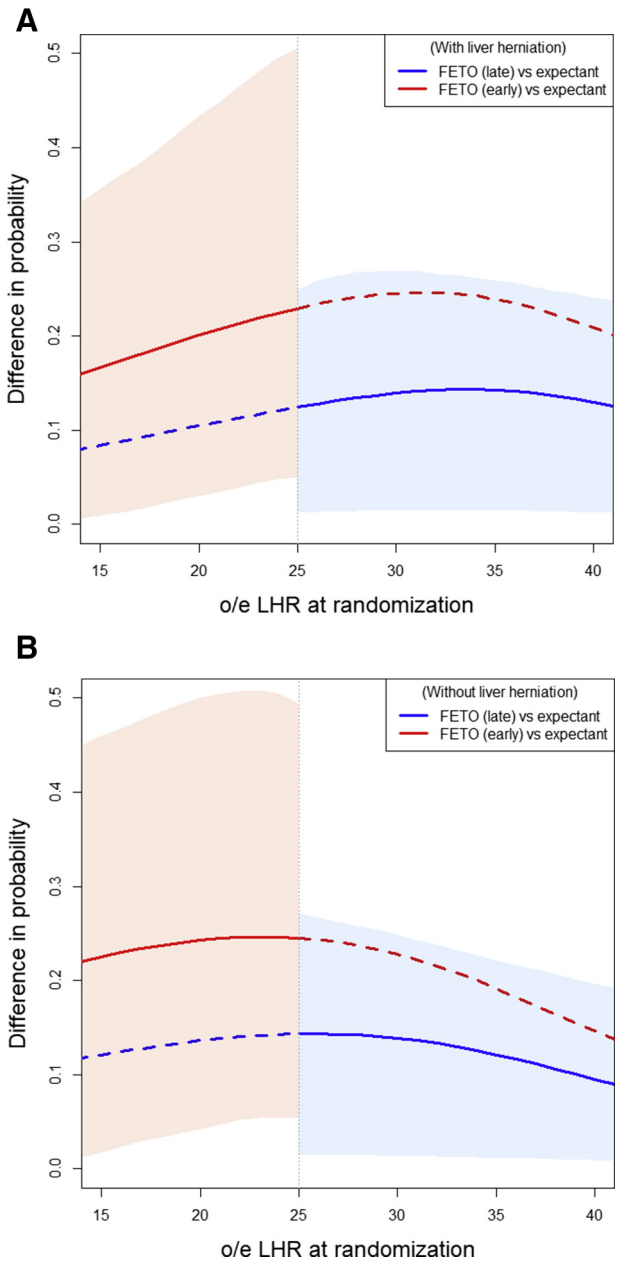
SUPPLEMENTAL FIGURE 2
**Estimated differences in the probability for survival to discharge for FETO vs expectant management**




Based on the model in Table 2. Panel **A** refers to cases with liver herniation and panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas based on the percentile bootstrap method with 10,000 bootstraps.

*FETO*, fetoscopic endoluminal tracheal occlusion; *TOTAL*, Tracheal Occlusion To Accelerate Lung growth.

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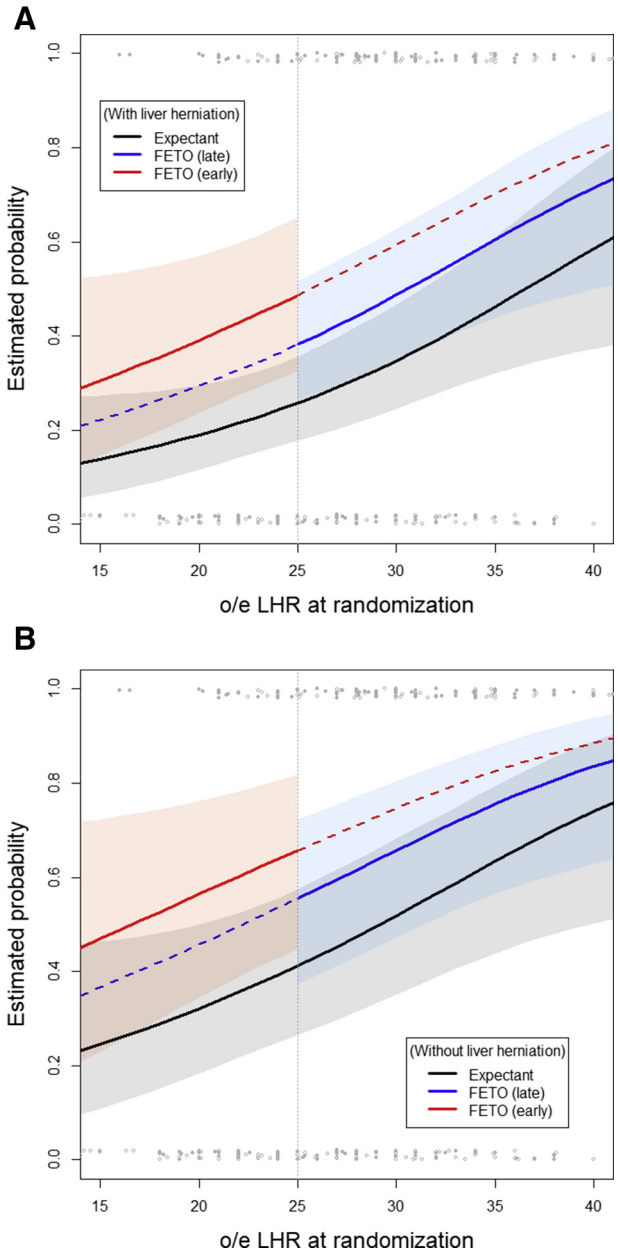
SUPPLEMENTAL FIGURE 3

**Estimated probabilities of survival to 6 months of age from the model in Table 3**




Panel **A** refers to cases with liver herniation, panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas. Raw patient data are indicated on top (survivors) and at the bottom (nonsurvivors) of the plot. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

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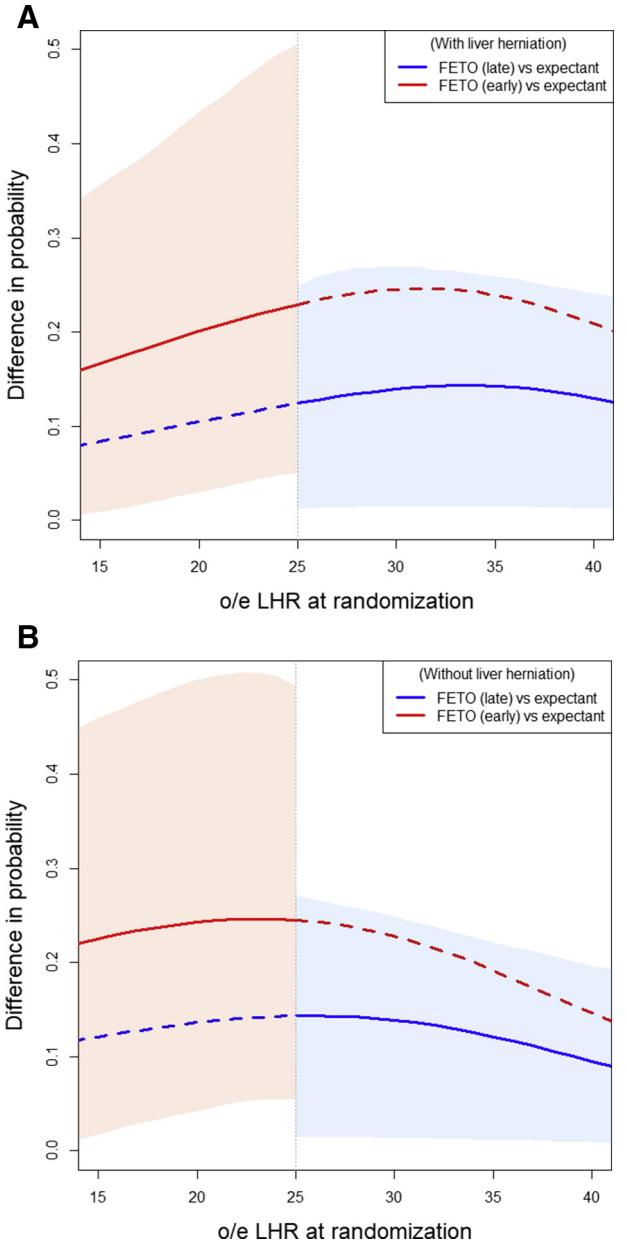
SUPPLEMENTAL FIGURE 4

**Estimated differences in the probability for survival to 6 months for FETO vs expectant management**




Panel **A** refers to cases with liver herniation, panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas based on the percentile bootstrap method with 10,000 bootstraps.

*FETO*, fetoscopic endoluminal tracheal occlusion; *TOTAL*, Tracheal Occlusion To Accelerate Lung growth.

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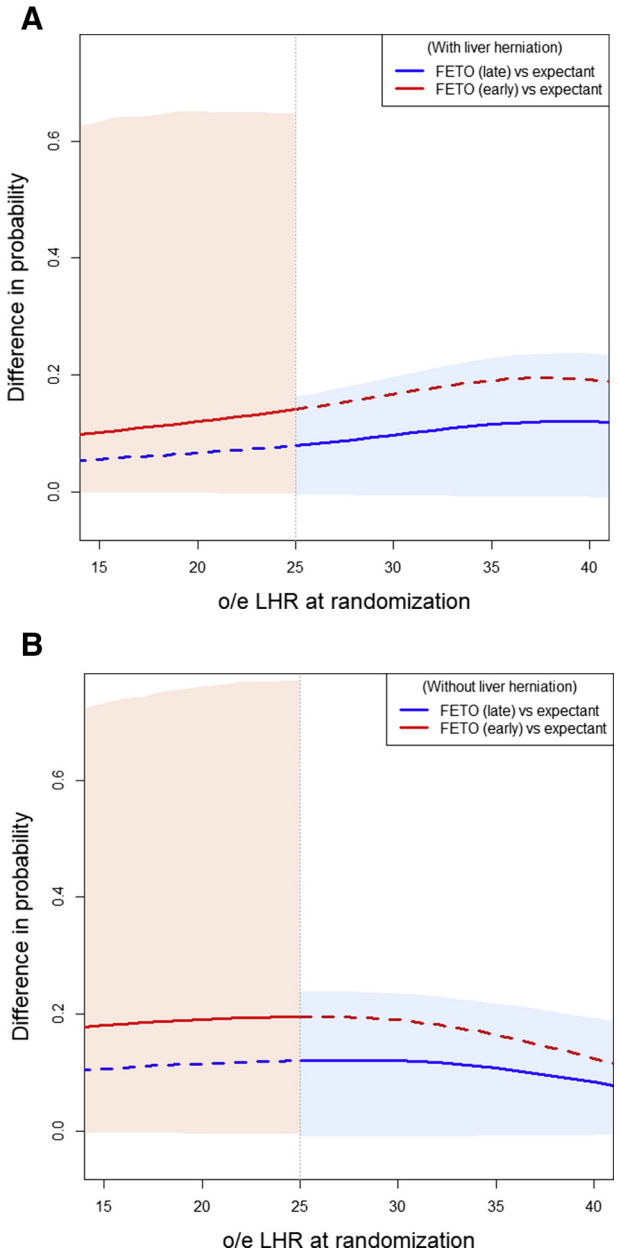
SUPPLEMENTAL FIGURE 5

**Estimated differences in the probability for survival to 6 months without oxygen supplementation for FETO vs expectant management**




Panel **A** refers to cases with liver herniation, panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas based on the percentile bootstrap method with 10,000 bootstraps.

*FETO*, fetoscopic endoluminal tracheal occlusion; *TOTAL*, Tracheal Occlusion To Accelerate Lung growth.

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SUPPLEMENTAL TABLE 1
**Patients per center and per trial**

| Center | FETO center location | Pooled, n (%) | RCT moderate, n | RCT severe, n |
|---|---|---|---|---|
| 1 | Leuven, Belgium | 77 (27) | 56 | 21 |
| 2 | Paris Antoine Béclère, France | 65 (23) | 46 | 19 |
| 3 | London, United Kingdom | 45 (16) | 18 | 27 |
| 4 | Barcelona, Spain | 35 (12) | 34 | 1 |
| 5 | Milan, Italy | 15 (5) | 10 | 5 |
| 6 | Bonn, Germany | 14 (5) | 13 | 1 |
| 7 | Toronto, Canada | 6 (2) | 0 | 6 |
| 8 | Brisbane, Australia | 5 (2) | 5 | 0 |
| 9 | Houston, Baylor College, United States | 5 (2) | 5 | 0 |
| 10 | Paris Necker, France | 5 (2) | 5 | 0 |
| 11 | Warsaw, Poland | 5 (2) | 1 | 4 |
| 12 | Tokyo, Japan | 5 (2) | 0 | 5 |
| 13 | Houston UTH, United States | 4 (1) | 2 | 2 |
| 14 | Rome, Italy | 1 (0.3) | 1 | 0 |
| | TOTAL | 287 (100) | 196 | 91 |

*FETO*, fetoscopic endoluminal tracheal occlusion; *RCT*, randomized controlled trial; *UTH*, The University of Texas Health Science Center.

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SUPPLEMENTAL TABLE 2

**Descriptive statistics of baseline characteristics and endpoints for the data from the trial on moderate hypoplasia**

| Baseline characteristics | Fetoscopic endoluminal tracheal occlusion (n=98) | Expectant (n=98) |
|---|---|---|
| Maternal age (y) | 31.1 (27.5–33.7) | 31.6 (27.4–34.9) |
| Gestational age (wk) | 28.4 (26.6–29.6) | 28.2 (27.0–29.3) |
| Nulliparous | 45 (46) | 48 (49) |
| Body mass index (kg/m$^2$) | 23.8 (20.4–28.2) | 22.2 (21.0–25.9) |
| Smoking during pregnancy | 11 (11) | 8 (8) |
| Alcohol use during pregnancy | 1 (1) | 1 (1) |
| Ethnicity | | |
| White | 87 (89) | 88 (90) |
| Asian | 3 (3) | 3 (3) |
| Black | 2 (2) | 2 (2) |
| Other | 6 (6) | 5 (5) |
| Ultrasound findings | | |
| Observed/expected lung-to-head ratio (%) | 30.9 (28.0–34.0) | 31.0 (28.0–34.5) |
| Liver herniation | 79 (81) | 78 (80) |
| Deepest vertical pocket of amniotic fluid (mm) | 6.0 (5.0–7.6) | 6.4 (5.5–7.7) |
| Cervical length (mm) | 37 (33–40) | 36 (31–40) |
| Placenta anterior | 50 (51) | 47 (48) |
| Placenta posterior | 43 (44) | 47 (48) |
| Placenta fundal | 5 (5) | 4 (4) |
| Endpoints | | |
| Survival to discharge (primary endpoint) | 62 (63) | 49 (50) |
| Survival to 6 mo of age | 61 (62) | 49 (50) |
| Survival to 6 mo without oxygen supplementation | 53 (54) | 43 (44) |
| Gestational age at live birth (wk) (n=191) | 35.9 (34.3–37.9) | 38.1 (37.0–38.9) |

Data are presented as median (interquartile range) or as number (percentage). There were no missing values for these parameters.

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SUPPLEMENTAL TABLE 3

**Descriptive statistics of baseline characteristics and endpoints for the data from the trial on severe hypoplasia**

| Baseline characteristics | Fetoscopic endoluminal tracheal occlusion (n=47) | Expectant (n=44) |
|---|---|---|
| Maternal age (y) | 32.1 (27.3–35.3) | 29.4 (25.9–33.8) |
| Gestational age (wk) | 27.7 (26.6–28.6) | 27.0 (26.4–28.0) |
| Nulliparous | 25 (53) | 20 (45) |
| Body mass index (kg/m$^2$) | 24.1 (21.9–29.7) | 24.3 (21.7–30.5) |
| Smoking during pregnancy | 0 (0) | 6 (14) |
| Alcohol use during pregnancy | 0 (0) | 0 (0) |
| Ethnicity | | |
| White | 37 (79) | 36 (82) |
| Asian | 6 (13) | 4 (9) |
| Black | 2 (4) | 4 (9) |
| Other | 2 (4) | 0 (0) |
| Ultrasound findings | | |
| Observed/expected lung-to-head ratio (%) | 21.0 (19.9–24.0) | 21.0 (18.0–23.0) |
| Liver herniation | 42 (89) | 38 (86) |
| Deepest vertical pocket of amniotic fluid (mm) | 6.5 (5.6–8.0) | 6.2 (5.8–7.4) |
| Cervical length (mm) | 34 (30–39) | 36 (32–39) |
| Placenta anterior | 33 (70) | 24 (55) |
| Placenta posterior | 13 (28) | 19 (43) |
| Placenta fundal | 1 (2) | 1 (2) |
| Endpoints | | |
| Survival to discharge (primary endpoint) | 17 (36) | 6 (14) |
| Survival to 6 mo of age | 17 (36) | 6 (14) |
| Survival to 6 mo without oxygen supplementation | 10 (21) | 3 (7) |
| Gestational age at live birth (wk) (n=88) | 34.6 (31.9–36.7) | 38.4 (36.6–39.0) |

Data are presented as median (interquartile range) or as number (percentage). There were no missing values for these parameters.

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## Appendix A

Additional information about sample size and statistical analysis.

### Change to the statistical analysis plan based on reviewers' comments

Because of a reviewer comment, the final approach for the regression models deviated from the initial approach. Initially, we did not include the trial covariate and aimed to adjust for fetal surgery center instead. The fetal surgery center is where the patient was assessed, randomized, and in the case of fetoscopic endoluminal tracheal occlusion, the balloon was also inserted and removed. Because randomization was not stratified, the individual trials did not require an adjustment for center. When pooling data, however, the distribution of fetal surgery center was different for moderate and severe cases. We did not adjust for center using a random intercept: many centers had few recruits, causing computational difficulties (no convergence). Instead, we used a categorical variable: center 1 (n=77), center 2 (n=65), and the other centers (n=145). We used only 3 levels to avoid spending too many model parameters on this covariate. We also applied variable selection on this covariate only (that is, other covariates were included by default) using an alpha level of 0.157. For all endpoints, the center covariate was omitted because the obtained *P* value was >.157. However, using a binary trial covariate to indicate in which trial each participant was recruited (severe vs moderate) may be a better approach to cover remaining heterogeneity between trials.

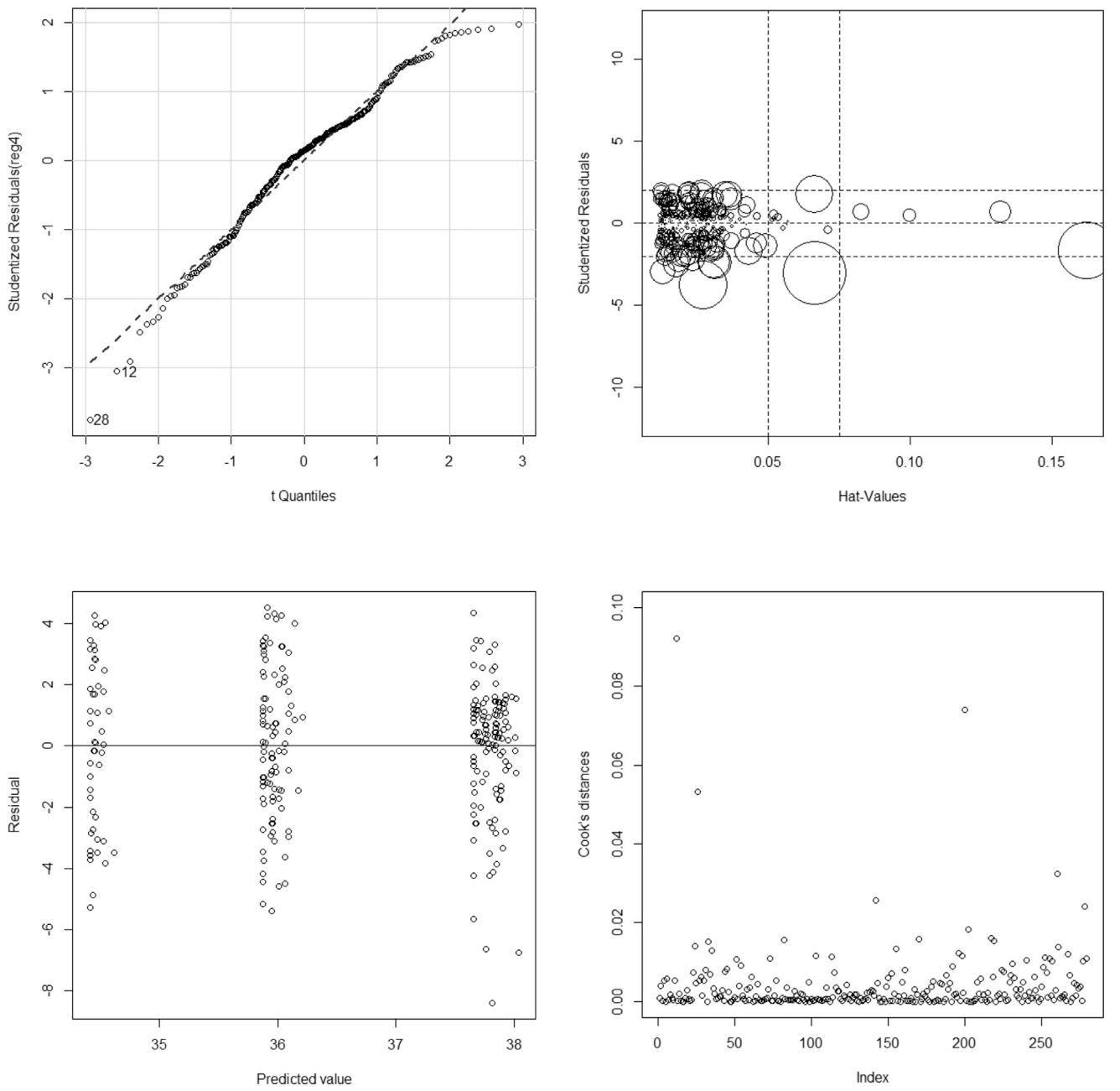
### Penalized regression

We used penalized maximum likelihood estimation where the penalty factor lambda is chosen using the corrected Akaike information criterion from a grid of value between 0 and 20 with steps of 0.1. We used the pentrace function from the rms R package.[12]

### Model diagnostics for the ordinary least squares regression model of gestational age at delivery

We state in the main text that GA at delivery does not need to be transformed. We examined the plots shown in Supplemental Figure 6 and Supplemental Figure 7 (after applying the optimal Box-Cox transformation for GA at delivery).

**SUPPLEMENTAL FIGURE 6**
**Diagnostic plots for the model presented in Table 5**




Top left presents the qq plot, top right the residual by predicted value plot to evaluate heteroskedasticity, bottom left the influence plot of studentized residuals by hat values, and bottom right the Cook's distance values for all observations.

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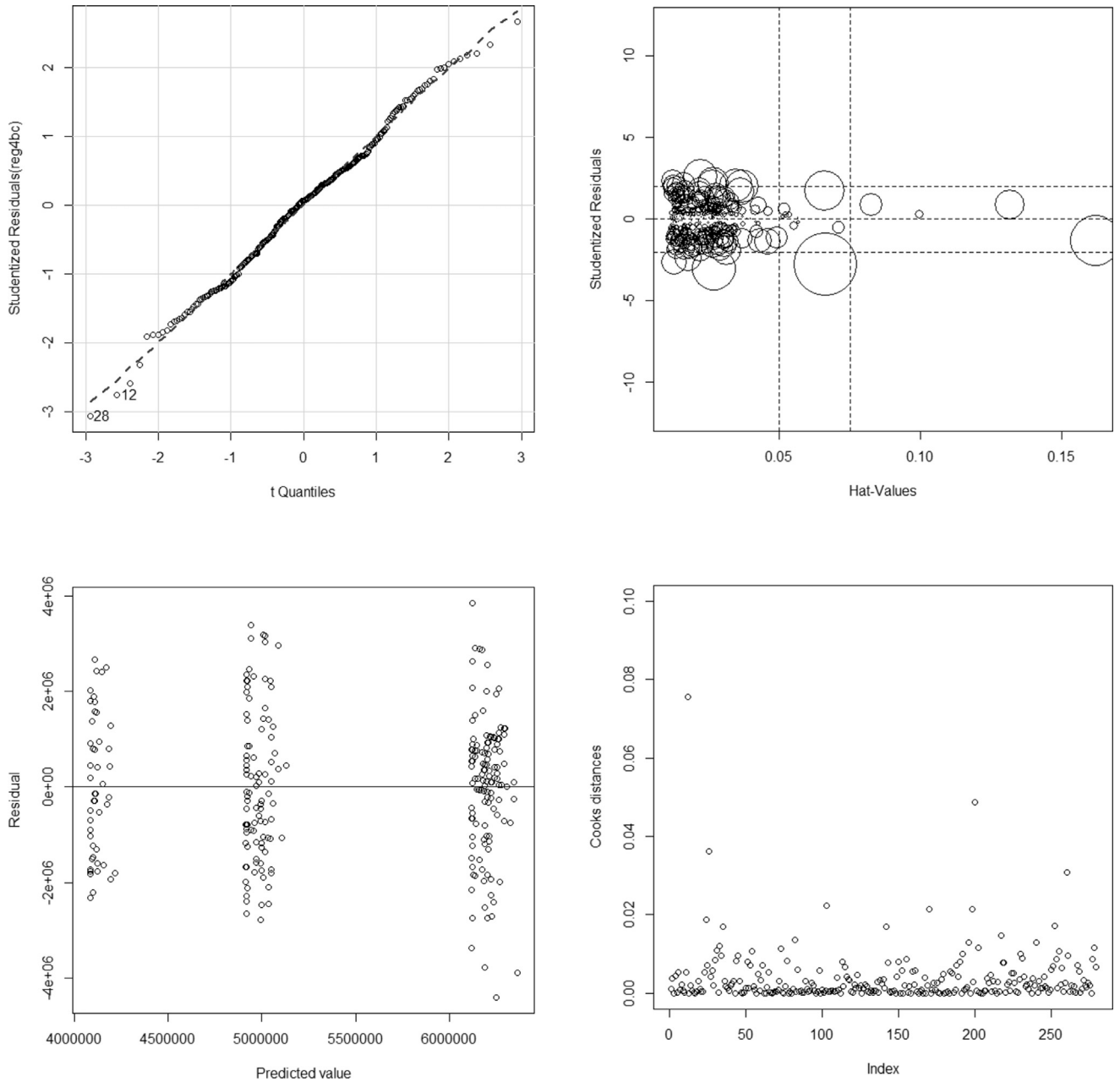
SUPPLEMENTAL FIGURE 7

**Diagnostic plots for the regression model of gestational age at delivery after the optimal Box-Cox transformation (lambda 4.727)**




The top left presents the qq plot, the top right represents the residual by predicted value plot to evaluate heteroskedasticity, the bottom left the influence plot of studentized residuals by hat values and bottom right the Cook's distance values for all observations.

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### Sample size

We considered 8 parameters in the multivariable analyses. The number of 'events' is defined by the number of cases in the smallest outcome category for binary endpoints and by the sample size for continuous endpoints. The number of 'events' was 134 for survival to discharge (134 survived, 153 did not survive), 133 for survival to 6 months, 109 for survival to 6 months without oxygen dependency, and 279 for gestational age at live birth. For the primary endpoint, there were 134/8=17 events per parameter. For survival to 6 months without oxygen dependency, there were 109/8=14 events per variable. Although the events per variable is an overly rough criterion that ignores contextual factors, these values are acceptable by most common standards when the focus is on model coefficients rather than on making robust predictions for new patients.[16] After changing the analysis plan following a reviewer comment, the multivariable analyses contained only 7 parameters and hence the number of events per parameter was higher.

## Appendix B

This section discusses the mediating role of preterm premature rupture of membranes (PPROM) for the effect of fetoscopic endoluminal tracheal occlusion on gestational age (GA) at live birth.

To study the potential mediating role of PPROM, we conducted a classical Baron-Kenny mediation analysis.[38] First, we repeated the analysis of GA at live birth, but added PPROM before 37 weeks as a binary covariate. We were interested in the effect of PPROM, and in the eventual decrease of the effects of intervention and early balloon insertion after adding PPROM as covariate (Supplemental Table 4). Relative to the model without PPROM (Table 5 in main paper), the coefficient of intervention reduced by 46% (from −1.70 to −0.91), the coefficient for early insertion reduced by 13% (from −1.51 to −1.32).

**SUPPLEMENTAL TABLE 4**
**Linear regression of gestational age at live birth (in weeks) with additional correction for PPROM before 37 weeks (n = 279)**

| Covariate | B (SE) | *P* value |
|---|---|---|
| Intervention: FETO (late insertion) vs expectant | −0.91 (0.28) | .0014 |
| Early balloon insertion (yes vs no) | −1.32 (0.46) | .0044 |
| PPROM <37 wk (yes vs no) | −2.63 (0.28) | <.0001 |
| o/e LHR (%) | −0.0067 (0.041) | .984 |
| o/e LHR, nonlinear | 0.0035 (0.044) | |
| Liver herniation (yes vs no) | 0.0026 (0.31) | .993 |
| Trial (severe vs moderate) | −0.042 (0.44) | .924 |

*B*, model coefficient; *FETO*, fetoscopic endoluminal tracheal occlusion; *o/e LHR*, observed/expected lung-to-head ratio; *PPROM*, preterm premature rupture of membranes; *SE*, standard error.
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Second, we conducted a logistic regression analysis of PPROM using the same covariates as in the other models (intervention, early balloon insertion, o/e LHR with nonlinear parameter, and liver herniation) (Supplemental Table 5).

**SUPPLEMENTAL TABLE 5**
**Logistic regression of preterm premature rupture of membranes <37 weeks (n = 279)**

| Covariate | B (SE) | *P* value |
|---|---|---|
| Intervention: fetoscopic endoluminal tracheal occlusion (late insertion) vs expectant | 1.48 (0.30) | <.0001 |
| Early balloon insertion (yes vs no) | 0.39 (0.48) | .417 |
| o/e LHR (%) | −0.0017 (0.036) | .590 |
| o/e LHR, nonlinear | −0.031 (0.041) | |
| Liver herniation (yes vs no) | 0.13 (0.35) | .718 |
| Trial (severe vs moderate) | −0.34 (0.46) | .460 |

*B*, model coefficient; *o/e LHR*, observed vs expected lung-to-head ratio; *SE*, standard error.
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These results suggest that the effect of FETO on GA at live birth is partially mediated by PPROM: PPROM is has an effect on GA at live birth and its inclusion in the model halves the coefficient of the intervention variable, and the intervention variable has an effect on PPROM. There was little evidence that the effect of early balloon insertion on GA at live birth was (partly) mediated by PPROM, but this may have been caused by the fact that we included PPROM as a binary variable.

The classical Baron-Kenny approach to mediation analysis may however be flawed in certain circumstances, such as when there is an interaction between exposure (FETO in this study) and mediator (PPROM).[39] We therefore conducted a causal mediation analysis using version 4.5.0 of the mediate package for R.[40] We allowed for a possible interaction between the exposure (FETO) and the mediator (PPROM), using robust standard errors and 1000 simulations using the quasi-Bayesian Monte Carlo method. However, we did not use penalized estimation for this analysis. The results confirm the results from the classical analysis, suggesting that 52% of the total effect of FETO on GA at live birth is mediated by PPROM (Supplemental Table 6). The evidence of an interaction between exposure and mediator was weak ($P$=.45).

**SUPPLEMENTAL TABLE 6**

**Linear regression of gestational age at live birth (in weeks) with additional correction for the preterm premature rupture of membranes before 37 weeks (n = 279)**

| Statistic | Estimate (95% confidence interval) |
|---|---|
| Total effect | −1.77 (−2.48 to −1.05) |
| Average causal mediated effect | |
| Expectant | −1.01 (−1.57 to −0.46) |
| FETO | −0.85 (−1.31 to −0.44) |
| Average | −0.93 (−1.38 to −0.48) |
| Average direct effect | |
| Expectant | −0.92 (−1.58 to −0.30) |
| FETO | −0.76 (−1.40 to −0.08) |
| Average | −0.84 (−1.45 to −0.21) |
| Proportion mediated (average) | 0.52 (0.31 to 0.84) |

*FETO,* fetoscopic endoluminal tracheal occlusion.

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## Appendix C

This section discusses the relationship between gestational age (GA) at fetoscopic endoluminal tracheal occlusion (FETO) and GA at live birth.

We observed that FETO and early balloon insertion had an effect on the gestational age (GA) at live birth, whereas there was very little evidence that the observed/expected lung-to-head-ratio and liver herniation were prognostic for GA at live birth. We therefore conducted a post-hoc simple linear regression of the GA at live birth on the gestational age at FETO in cases randomized to FETO and in whom FETO was performed (n=138, including the patient where balloon insertion failed because of the fetal position). We again used restricted cubic splines to model the GA at FETO (Supplemental Table 7, Figure 5 in main text).

**SUPPLEMENTAL TABLE 7**

**Linear regression of gestational age at live birth (in weeks) on gestational age at FETO in patients randomized to FETO and who received FETO (n = 138)**

| Covariate | B (SE) | *P* value |
|---|---|---|
| GA at FETO (wk) | 0.80 (0.33) | .017 |
| GA at FETO (wk), nonlinear | −0.20 (0.31) | .536 |
| Trial (severe vs moderate) | −0.083 (0.65) | .899 |

*B*, model coefficient; *GA*, gestational age; *FETO*, fetoscopic endoluminal tracheal occlusion; *SE*, standard error.

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There was evidence of a relationship between the gestational age (GA) at FETO and the GA at live birth, but considerable interindividual variability was observed. This is evident from Figure 5 (large spread of GA at live birth conditional on gestational age at FETO), and by the adjusted R-squared value of 11%. The R-squared value estimates the amount of variability in the GA at live birth that is explained by variability in the GA at FETO.

An analysis including only a linear term for gestational age at FETO yielded a regression coefficient (beta) of 0.59 (standard error, 0.23), suggesting that live birth is delayed by on average 4 days ($7 \times 0.59 = 4.1$; 95% confidence interval, 1.0−7.3) per week delay in balloon insertion.