Figure

Amniotic lamellar body count and congenital diaphragmatic hernia in humans and in a rat model

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BACKGROUND: We examined the extent of fetal lung hypoplasia and lung maturation using the amniotic lamellar body count (LBC) in congenital diaphragmatic hernia (CDH).

METHODS: We obtained 30 amniotic fluid samples from human CDH cases during cesarean section. We assessed LBC, magnetic resonance imaging (MRI), and ultrasound findings for predicting the prognosis of CDH. We collected newborn amniotic fluid and lung tissue at embryonic day (E)21 from normal and nitrofen-induced CDH rats (administered 100 mg orally at E9). Amniotic LBCs in rats were measured using light microscopy.

RESULTS: In human CDH, LBC was significantly higher in the surviving than in the deceased group ($P < 0.01$). A significant positive correlation was observed between LBC and percentage of fetal lung volume on MRI ($P < 0.001$; $r = 0.716$). In rats, LBC was significantly higher in controls than in CDH rats ($P < 0.01$) and correlated with fetal lung weight.

CONCLUSION: We conclude that LBC is useful for predicting lung hypoplasia in human CDH after 35 gestational weeks and in a rat model of nitrofen-induced CDH.

Congenital diaphragmatic hernia (CDH) is a developmental discontinuity of the diaphragm that affects one in 2,500–5,000 live births (1), resulting in pulmonary hypoplasia and pulmonary hypertension, which are often fatal. To improve clinical care and counseling, reliable prenatal parameters predicting fetal outcome in CDH are urgently needed. Prenatal predictors of fetal outcome in CDH have been reported to be focused mainly on the assessment of fetal lung volume (FLV). Ultrasound and magnetic resonance imaging (MRI) have been used to measure antenatal FLV and to qualify fetal pulmonary hypoplasia, but a gold-standard parameter has not yet been established.

The lamellar body count (LBC) was first described in 1989 (2). Lamellar bodies are made up of a surfactant-containing lamellated structure and are secreted by type II pneumocytes (3). The similarity of lamellar body size to platelet size permits the use of a standard automated hematologic cell counter to quantify the number of lamellar bodies in amniotic fluid (4). Thus, the LBC is used to predict the degree of fetal lung maturity (FLM). Several studies have shown the LBC to accurately predict FLM (5–7). The LBC can be measured quickly and inexpensively, such that it is equal to or even better than the lecithin-to-sphingomyelin ratio for predicting the occurrence of respiratory distress syndrome (7).

In the current study, we hypothesized that if fetal pulmonary hypoplasia occurs because of CDH, the number of amniotic lamellar bodies secreted from the fetal lung into the amniotic cavity would be small. Therefore, we examined the LBC value in CDH neonates and confirmed it to be a useful predictor of CDH severity. Furthermore, given that LBC was originally a predictor of FLM, in the current study we also examined whether fetal lung maturation in CDH neonates is equivalent to that in normal neonates, using a rat CDH model.

RESULTS

Registration of Clinical CDH Cases

We initially enrolled 30 cases diagnosed with CDH prenatally (Figure 1). We excluded 11 cases (delivery before 35 weeks: $n = 5$; amniotic sampling failure: $n = 3$; trisomy 18: $n = 2$; neonatal death due to severe congenital heart disease: $n = 1$). Therefore, 19 cases were ultimately analyzed. We divided the newborns into two groups: infants who survived ($n = 16$) and those who died ($n = 3$).

Prognostic Findings for CDH in Humans

Background and prenatal findings of our CDH cases are shown in Table 1. LBC was significantly higher in the surviving group than in the deceased group ($1.6 \pm 0.7 \times 10^4/\mu l$ vs. $0.5 \pm 0.3 \times 10^4/\mu l$; $P < 0.01$). The percentage of FLV on MRI was also significantly higher in the surviving than in the deceased group ($36.6 \pm 27.2 \times 10^4/\mu l$ vs. $11.3 \pm 6.14 \times 10^4/\mu l$; $P < 0.05$). We assessed LBC and percentage of FLV as potentially useful parameters for predicting neonatal nitric oxide (NO) ($n = 12$) and extracorporeal membrane oxygen (ECMO) ($n = 4$) requirements in the surviving group ($n = 16$). LBC was significantly lower in infants needing NO and ECMO therapies than in those not needing such therapies ($1.5 \pm 0.6 \times 10^4/\mu l$ vs. $2.4 \pm 0.4 \times 10^4/\mu l$ in NO; $P = 0.010$, and $1.2 \pm 0.4 \times 10^4/\mu l$ vs. $1.9 \pm 0.6 \times 10^4/\mu l$ in ECMO; $P = 0.049$, respectively). The percentage of FLV on MRI was significantly lower in infants needing NO and ECMO therapies than in those not needing such therapies (24.4 ± 11.7 vs. 73.2 ± 28.3 for NO;

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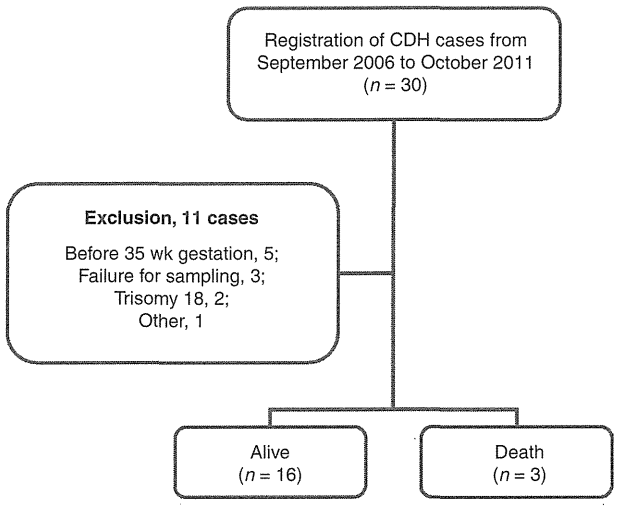

Figure 1. Algorithm for inclusion and exclusion of cases in this study. "Other" refers to a case of neonatal death due to severe congenital heart disease. CDH, congenital diaphragmatic hernia.

Table 1. Background and prenatal findings of congenital diaphragmatic hernia in humans

	Patients who survived ($n = 16$)	Patients who died ($n = 3$)	P value
Maternal age (y)[a]	29.0 ± 4.7	31.7 ± 2.1	0.357
GA at delivery (weeks)[a]	37.44 ± 0.13	36.33 ± 0.67	0.054
Birth weight <2,500 g[b]	2/16 (12.5%)	0/3 (0%)	0.517
Male[b]	11/16 (69%)	2/3 (67%)	0.943
DM[b]	0/16 (0%)	0/3 (0%)	NA
PIH[b]	0/16 (0%)	0/3 (0%)	NA
FGR[b]	1/16 (6%)	0/3 (0%)	0.517
GA at diagnosis (wks)[a]	28.6 ± 3.3	25 ± 1.0	0.086
LBC (10^4/μl)[a]	1.6 ± 0.7	0.5 ± 0.3	0.009
LHR[a]	1.39 ± 0.59	1.20 ± 0.40	0.233
FLV on MRI (cm^3)[a]	15.4 ± 11.2	7.28 ± 2.42	0.240
Percentage of FLV on MRI[c]	36.6 ± 27.2	11.3 ± 6.14	0.034
Liver herniation[b]	7/16 (44%)	3/3 (100%)	0.466
Positive CPB[b]	14/16 (88%)	0/3 (0%)	0.084
Polyhydramnios[b]	3/16 (19%)	3/3 (100%)	0.222

CPB, contralateral pulmonary baseline on MRI; DM, diabetes mellitus; FGR, fetal growth restriction; FLV, fetal lung volume; GA, gestational age; LBC, lamellar body count; LHR, lung-to-head ratio; MRI, magnetic resonance imaging; NA, not available; PIH, pregnancy-induced hypertension.

[a]Student's t-test was used. [b]χ^2 test was used. [c]Mann–Whitney U test was used.

$P = 0.002$, and 13.9 ± 7.5 vs. 44.2 ± 27.2 for ECMO; $P = 0.008$, respectively). Other prenatal predictors including gestational age at diagnosis, lung to head ratio (LHR), absolute FLV, liver herniation, and polyhydramnios did not significantly impact the outcomes of CDH infants. The rates of diabetes mellitus, preeclampsia, and fetal growth restriction did not differ significantly between the two groups. Furthermore, we analyzed the relationship between LBC and percentage of FLV (Figure 2). A significant positive correlation was observed ($P < 0.001$; $r = 0.716$).

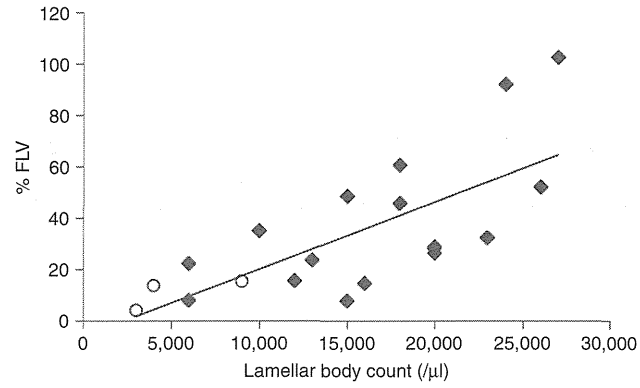

Figure 2. Scattergram showing the relationship between percentage of FLV and lamellar body count in human CDH. A significant positive correlation was observed ($P < 0.001$; $r = 0.716$). Filled diamonds indicate patients who survived, open circles indicate patients with CDH who died. CDH, congenital diaphragmatic hernia; FLV, fetal lung volume.

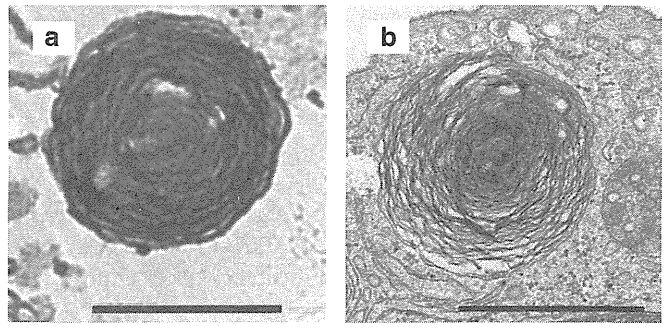

Figure 3. Electron microscopy of a lamellar body. Lamellar bodies are detected in (**a**) amniotic fluid and (**b**) type II alveolar cells. The lamellar body sizes were similar, at ~1 μm, in the two sites. Bars = 1.0 μm.

LBC and Lung Weight in a Rat CDH Model

In the rat experiment, we collected amniotic fluid and lung tissue from newborns at embryonic day (E)21 and confirmed the presence of lamellar bodies in both the amniotic fluid (Figure 3a) and type II alveolar cells of the lung (Figure 3b) using electron microscopy. We also confirmed a similar lamellar body size in amniotic fluid and type II alveolar cells, of approximately 1–2 μm, comparable to the lamellar body findings in human amniotic fluid (2,8). Furthermore, after exposure of pregnant rats to a 100-mg dose of nitrofen, 48.8% (61 of 125) of newborns had diaphragmatic hernias. LBC was significantly higher in control rats than in CDH rats (3,527 ± 211/μl vs. 1,564 ± 358/μl; $P < 0.01$) (Figure 4a). Furthermore, lung weight was also significantly higher in controls than in CDH newborn rats (0.143 ± 0.024 g vs. 0.071 ± 0.022 g; $P < 0.01$) (Figure 4b). In addition, the LBC per unit lung weight in controls was similar to that in CDH rats (25.3 ± 1.59 × 10^3/μl vs. 34.6 ± 15.2 × 10^3/μl; $P = 0.544$).

Western Blotting for ABCA3 Expression in Fetal Rat Lung

We examined the expression of ABCA3 in lung tissues from newborn rats, delivered at E21, by western blotting (Figure 5a). ABCA3 expression did not differ significantly between controls and CDH rats ($P = 0.551$) (Figure 5b).

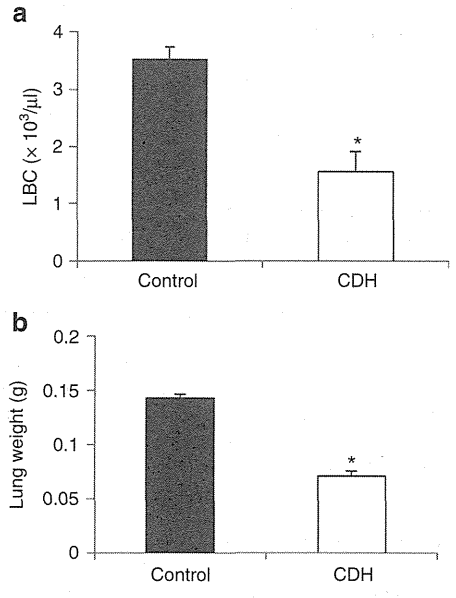

Figure 4. Amniotic fluid LBC and lung weights in newborn rats. (**a**) LBC was significantly higher in controls than in CDH rats (3,527 ± 211/μl vs. 1,564 ± 358/μl; $P < 0.01$). (**b**) Furthermore, newborn lung weights were significantly higher in controls than in CDH rats (0.143 ± 0.024 g vs. 0.071 ± 0.022 g; $P < 0.01$). *$P < 0.01$. Bars represent SEM. Controls are normal pups. CDH, congenital diaphragmatic hernia; LBC, lamellar body count.

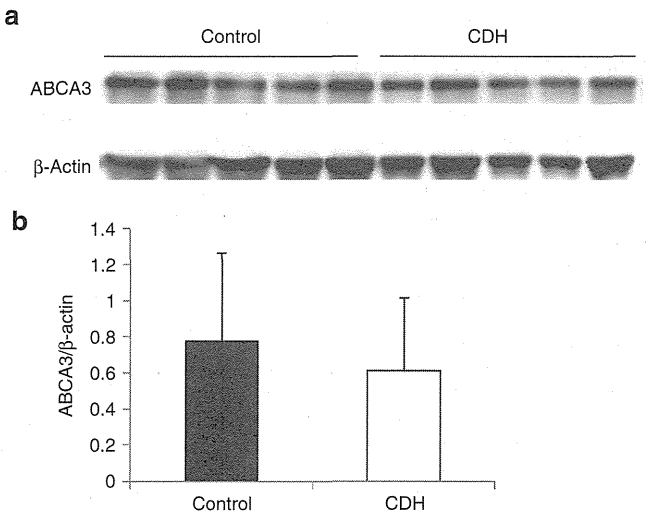

Figure 5. ABCA3 and β-actin expressions in rat lung tissue. (**a**) Western blotting and (**b**) the ratio of ABCA3 expression to that of β-actin revealed that ABCA3 expression did not differ significantly between controls and CDH rats ($P = 0.551$). Bars represent SEM. Controls are normal pups. ABCA3, ATP-binding cassette transporter A3; CDH, congenital diaphragmatic hernia.

DISCUSSION

Pulmonary hypoplasia accounts for significant mortality and morbidity in neonates with CDH. Therefore, a precise predictor of FLV is required. In the current study, we found that LBC and percentage of FLV on MRI differed significantly between surviving and deceased CDH cases. Conversely, LHR did not differ significantly. LHR is reportedly useful for predicting subsequent survival (9), but some recent studies could not confirm the prognostic value of LHR for survival or the necessity of ECMO therapy (10,11). In general, ultrasonography has certain disadvantages. Image resolution is sometimes limited by fetal position, maternal obesity, and oligohydramnios, which result in data variability among examiners. This can make it difficult to distinguish between fetal lung and liver on ultrasound. The FLV prediction using MRI has been reported to be accurate (12,13), but absolute FLV alone is not predictive of outcome because increasing lung volume with increasing gestational age could be a confounding factor (14). Therefore, percentage of FLV on MRI is considered to be a more useful predictor in CDH (15). In the current study, percentage of FLV on MRI, not absolute FLV, differed significantly in terms of the prediction of mortality in CDH. We examined factors such as liver herniation, complete contralateral pulmonary baseline on MRI, and polyhydramnios but found no significant differences, although positive findings for these factors correlated with a poor outcome (death in all cases).

Amniotic LBC is reportedly an accurate predictor of FLM and has the advantage of being able to be determined as an absolute value on the basis of objective application of a cell counter. In the current study, we found that LBC correlated significantly with neonatal mortality in CDH after 35 gestational weeks. Moreover, LBC correlated closely with percentage of FLV on MRI. From this viewpoint, we hypothesize that LBC might be useful not only as a predictor of mortality but also as a predictor of hypoplastic lung in CDH after 35 gestational weeks. The lamellar body is a surfactant-containing lamellated structure that is secreted by type II pneumocytes (3). Therefore, LBC is used to estimate surfactant production *in utero* and, if lung maturation has been achieved in the fetus, to predict the extent of FLV.

Several studies have shown that diaphragmatic defects could be induced by administering a single 100-mg dose of nitrofen to pregnant rats between E8 and E11, and most significantly, that the defects produced were remarkably similar to those documented in human Bochdalek CDH with respect to their size, location, and accompanying intrusion of the abdominal viscera into the thoracic cavity (16–18). We administered a single 100-mg dose of nitrofen at E9 and confirmed the diaphragm defects in 48% of the rat pups. This rate is acceptable as compared with those in other reports (19,20).

In a rat model, we confirmed the presence of lamellar bodies in amniotic fluid as well as in alveolar type II cells using electron microscopy; we also confirmed that the size of these lamellar bodies was similar to that in humans. In human samples, we were able to examine amniotic LBCs using a hematologic cell counter in our hospital, but we could not apply this device to examine the lamellar bodies in the rat model. Hence, we counted rat lamellar bodies by light microscopy. LBCs determined by light microscopy reportedly correlate well with results obtained using standard hematologic cell counter techniques in human samples (21). This is the first report demonstrating amniotic LBCs in a rat model.

In our CDH rat model, fetal lung weight was significantly decreased, and amniotic LBC was also significantly decreased

as compared with controls. The LBC per unit lung weight in CDH was similar to that in controls. Therefore, we can conclude that amniotic LBCs show a good correlation with fetal lung weight in CDH rat models, similar to the human CDH results. Furthermore, we examined ABCA3 expression by western blotting to assess fetal lung maturation in the rat. ABCA3 protein is expressed predominantly at the limiting membranes of the lamellar bodies in alveolar type II cells (22) and plays an essential role in pulmonary surfactant lipid metabolism, lamellar body biogenesis, surfactant protein-B processing, and lung development late in gestation (23). In the current study, ABAC3 expressions were similar in CDH and non-CDH pups at full term. Controversy persists regarding whether or not humans with CDH and animal models of CDH have a surfactant deficiency. Our study revealed that fetal lung maturation was not delayed in CDH as compared with non-CDH in both humans and the rat nitrofen-induced model of CDH, observations that are consistent with recently reported results (1,24,25).

Our study has limitations. First, the CDH sample number for the human data was small. To evaluate the efficacy of LBC for predicting lung hypoplasia in CDH, a larger study would be necessary. Next, we must focus on the interpretation of LBC before 35 gestational weeks. LBC was originally a useful predictor for FLM, such that we could not evaluate lung hypoplasia using LBC when the fetal lung was immature. Before 35 gestational weeks, MRI is recommended for predicting lung hypoplasia in CDH. Finally, we dealt with an animal model of lung hypoplasia in CDH based on the teratogenic effects of nitrofen. A surgical model is potentially suitable for investigating interventional strategies in CDH, and further study would thus be necessary.

In conclusion, LBC is potentially useful for predicting hypoplastic lung in human CDH after 35 gestational weeks. LBC has advantages because it can be measured quickly and inexpensively, and it can be objectively determined as an absolute value using a cell counter. This is the first report predicting outcomes of CDH using LBC. Our results also addressed the fact that fetal lung maturation is not delayed in CDH as compared with controls for human CDH and for the nitrofen-induced rat CDH model. Although this study has limitations, we believe that amniotic LBC can serve a new tool for predicting the outcomes and the necessity of neonatal respiratory management of CDH and would therefore be useful for prenatal counseling.

METHODS

Case Registration and Amniotic Fluid Collection in Clinical CDH

Data were collected from October 2006 to October 2011 at Nagoya University Hospital, Nagoya, Japan. Thirty amniotic fluid samples from CDH cases were obtained with signed informed consent. All amniotic fluid samples were obtained at cesarean section performed at 27–38 gestational weeks. We collected amniotic fluid by centesis while looking directly at the amniotic bag after incision of the myometrium during cesarean section. Samples were analyzed immediately after arrival at the laboratory without centrifugation, according to a standardized methodology for LBC reported by Neerhof *et al.* (6). The LBC (per microliter) was determined using a platelet channel on the Sysmex XE-2100 (Sysmex, Kobe, Japan), and the procedure took no more than 30 min. At least 1 ml of amniotic fluid is needed to measure LBC. This study was approved by the ethics committee of Nagoya University Hospital.

Neonatal data such as gestational age at delivery, birth weight, sex, and prognosis including respiratory management (NO and ECMO) were extracted. Fetal growth restriction was defined as birth weight less than the mean − 1.5 SD for gestational age in Japan (26).

Clinical characteristics of the mother such as preeclampsia and diabetes mellitus, both preexisting and gestational (27), were documented for the assessment.

Prognostic Factors in Clinical CDH

The LHR is the ratio of the right lung area to the head circumference, as described by Metkus *et al.* (28).

We calculated the percentage of right sided FLV, defined as the ratio of the observed right FLV to the expected right FLV, using MRI (Visart EX 1.5T; Toshiba, Tokyo, Japan), as described by Hayakawa *et al.* (15). We also evaluated whether the right-sided pulmonary baseline was completely present. Magnetic resonance images with a complete baseline on at least one section were considered to have a complete pulmonary baseline. This finding has been reported to correlate with the severity of CDH (15). Polyhydramnios was defined as an amniotic fluid index of >25 (29).

Nitrofen-Induced Rat CDH Model

All animal experimental procedures were performed in accordance with the Nagoya University institutional guidelines for animal care, which conform to the National Institutes of Health animal care guidelines. Timed pregnant Sprague–Dawley rats were purchased from Chubu Kagaku Shizai (Nagoya, Japan). Rats were exposed to nitrofen (Sigma-Aldrich, Tokyo, Japan) on day 9 of pregnancy. Nitrofen (100 mg) was dissolved in 1 ml of olive oil. A rigid metal tube was then inserted into the rat's esophagus to administer the olive oil–nitrofen mixture to the stomach. At E21, we performed cesarean section and collected amniotic fluid and lung tissue from the newborns of both normal rats and those in which CDH had been induced by nitrofen.

Transmission Electron Microscope

Materials in fixing solution were centrifuged for 2 min at 4,000 rpm and then for 4 min at 6,000 rpm. Materials were washed three times in 0.1 mol/l phosphate buffer (pH 7.4) for a duration of 10 min each time and then postfixed with 1% osmium tetroxide for 1 h. After dehydration with a series of graded ethanol concentrations, ultrathin sections were cut vertically to the epidermal surface on an Ultracut S ultramicrotome (Reichert, Depew, NY). Sections were stained with uranyl acetate and lead citrate and examined with a JEOL-1400EX transmission electron microscope (Jeol, Tokyo, Japan).

Amniotic LBC in the Rat

We measured amniotic LBC using light microscopy in the rat model, as described by Hunter *et al.* (21). In summary, a disposable plastic pipette was used to charge amniotic fluid onto a Burker-Turk hemocytometer (ERMA, Tokyo, Japan). The hemocytometer was viewed at ×400, and lamellar bodies were counted for each of the four corners of the Burker-Turk hemocytometer center grid, and the LBC was then divided by 4.

Western Blotting

Lung tissues from newborn rats were conserved at −80 °C in RNAlater (Qiagen, Tokyo, Japan). Then protein was extracted using protease inhibitor mixture tablets (Roche, Tokyo, Japan). The following antibody dilutions were used for western blot analysis: anti-ABCA3 (antimouse, incubated at 1:1,000 dilution; Covance, Tokyo, Japan), antimouse immunoglobulin G secondary antibodies (Cell Signaling Technology, Tokyo, Japan), and anti-β-actin (antimouse, incubated at 1:5,000 dilution; Abcam, Cambridge, UK). ABCA3 signals were normalized to β-actin signals for quantification.

Statistical Analysis

Statistical analysis of the data was performed with SPSS for Windows (V.19.0; SPSS, Chicago, IL) and Excel for Windows 2010 (Microsoft, Redmond, WA). Differences between the means were assessed using the Mann–Whitney–Wilcoxon test for unpaired samples. Wherever appropriate, Student's *t*-test was also used. A P value <0.05 was considered significant.

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STATEMENT OF FINANCIAL SUPPORT

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ORIGINAL ARTICLE

Valved shunt as a treatment for obstructive uropathy: does pressure make a difference?

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Abstract
Purpose A valved ventriculo-peritoneal shunt (V-P shunt) as a vesico-amniotic shunt (V-A shunt) preserves the filling/emptying cycle and normal bladder development in fetal lambs with bladder outlet obstruction. The optimal pressure for such shunts is unknown.
Materials and methods We created obstructive uropathy in 60-day gestation fetal lambs. A V-A shunt was placed 3 weeks later, using a low-pressure (Group L: 15–54 mmH_2O) or a high-pressure (Group H: 95–150 mmH_2O) V-P shunt. We included non-shunted (obstructive uropathy, Group O) and control lambs (Group C). All were delivered at 130 days. Bladder volumes, bladder thickness, renal and bladder histology were compared.
Results Seventeen lambs had an obstructive uropathy created. Five Group L (four survived), four Group H (three survived) and five Group O survived. Body weight and crown-to-rump lengths of the three groups were not significantly different. Group H lambs had a dilated urachus, urinary ascites and severe ureteral dilatation similar to Group O lambs. There were four Group C lambs. Bladder volume was 10, 15 and 1,150 ml in Group H, 115 ± 67.9 ml in Group L, 128 ± 99.8 ml in Group O and 24.5 ± 3.84 ml in Group C. Unlike Group O lambs, Group L did not have urinary ascites, urinomas or renal dysplasia.
Conclusion Low-pressure shunts preserved both bladder volume and renal development. High-pressure shunts did neither.

Keywords Obstructive uropathy · Fetal surgery · Urinoma · V-P shunt

Introduction

In utero shunting of obstructive uropathy was one of the first attempts at fetal therapy after the establishment of fetal transfusions for Rh isoimmunization [1–3]. It became a common treatment for obstructive uropathy but the indications and the optimal method of treatment are still not clearly established [4]. Our recent studies demonstrated that a vesico-amniotic shunt for obstructive uropathy in fetal lambs produced a thick-walled poorly compliant bladder [5–7]. We found that the placement of a valve shunt into an obstructed bladder resulted in a much more compliant bladder with reduced fibrosis in the bladder wall. A pressure controlled V-P shunt tube is able to decompress the bladder while also preserving its volume and reduce both muscle hypertrophy and interstitial fibrosis [8, 9]. We decided to attempt to define the optimal pressure to use when shunting an obstructed fetal bladder.

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Materials and methods

After approval was obtained from the Wellington School of Medicine and Health Sciences, University of Otago, Wellington Animal Ethics Committee (Application 02-11), timed gestation ewes (60 days' gestation) were transported from the farm 24 to 48 h before operation. They were examined by ultrasound to confirm the pregnancy and avoid unnecessary operations. The preoperative management and anesthetic and surgical techniques have been previously reported [10, 11]. The ewes were fasted for 24 h but allowed free access to water preoperatively. Creation of obstructive uropathy in fetal lambs at 60-day gestation was achieved by ligating the penile urethra and the urachus in male lambs and ligating the bladder neck and the urachus in female lambs, using fine SILASTIC tubing (Dow Corning, Midland, MI, USA) of 0.012 in. (0.3 mm) (Fig. 1).

The fetuses were then returned to the uterus, and the uterus and the ewe's abdomen were closed with monofilament absorbable suture (Polyglycoma: Covidien Japan Inc.) as we have previously described [11, 12]. Three weeks after creating the obstruction, the ewes were again anaesthetized, the uterus was reopened using the previous incision and the fetuses were exposed. The fetus's lower extremities and abdomen were delivered through the uterus, and a small hole was made in the bladder [13]. A purse string suture was placed in the bladder wall, and the peritoneal end of a Pudenz catheter was inserted into the bladder [7, 8]. In the low-pressure group (Group L) an Integra Neuro Sciences, Pudenz Peritoneal Catheter, Plainsboro, NJ, REF NL850-1380, (pressure of 15–54 mmH_2O) catheter was used. In the second group (Group H) a high-pressure Pudenz catheter, Integra NeuroSciences, Pudenz Peritoneal Catheter, Plainsboro, NJ, REF NL850-1382, (pressure of 95–150 cmH_2O) catheter was used in same fashion (Fig. 2). In the third group (Group O), no shunt was placed. The fetuses were returned to the uterus, and the uterus and ewe's abdominal wall were closed with monofilament absorbable sutures. Normal unoperated 130-day gestation lambs (Group C) were used as controls.

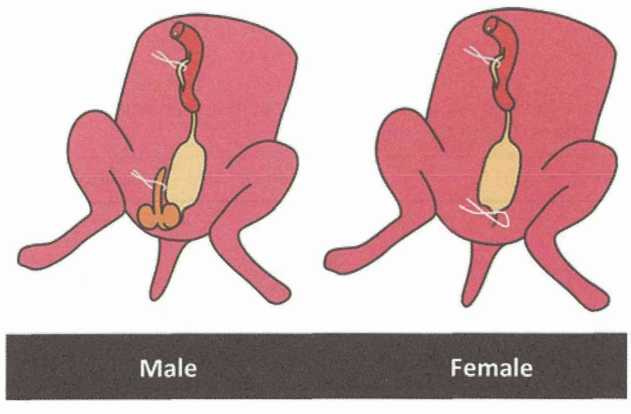

Fig. 1 Ligating the penile urethra and the urachus in male lambs and ligating the bladder neck and the urachus in female lambs

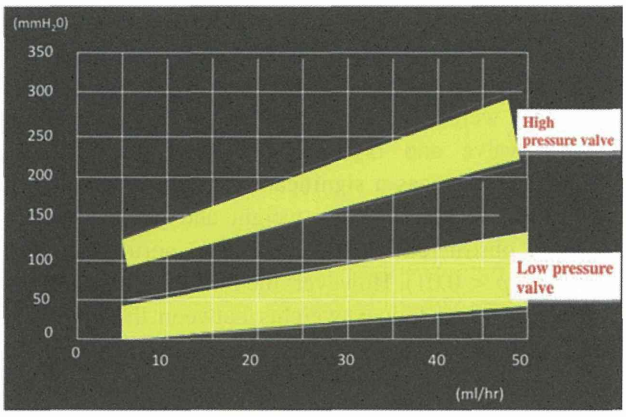

Fig. 2 The pressure and volume flow curve of the shunt tubes. The low-pressure catheter opens at 15–54 mmH_2O pressure if the urine flow is approximately 9 ml/h. The high-pressure catheter opens at 105–140 mmH_2O pressure at the same urine flow

At 130 days, the ewes were anesthetized and the fetuses were delivered by cesarean section. The lambs were then sacrificed using pentobarbital injected into the umbilical vein. The initial bladder volume was measured by aspirating the urine through the catheter in fetuses with shunts or through the bladder wall in normal or non-shunted obstructed lambs. The bladder volume was measured by infusing formalin through the shunt tube or the aspiration cannula until a pressure of 30 cmH_2O was achieved. The bladders were fixed distended with formalin at a pressure of 30 cmH_2O. The lamb's entire renal tract was removed en bloc and initially fixed in formalin. Samples were taken from the kidneys, ureters, and the bladder wall. Histologic sections of the bladder wall were stained with hematoxylin and eosin. We measured the distance from the base of the mucosa to the outmost fibers of the muscle layer in the bladder wall. Statistical comparisons of the multiple data were evaluated by the Dunnett test.

Results

Effective obstruction of both urethra and urachus was achieved in 16 fetuses and 14 survived (87 %). Nine fetuses were shunted using a valve shunt: five with a low-pressure valve (four of which survived) and four with a high-pressure valve (three of which survived). The shunts remained in place and were effective till 130 days of gestation. We obtained effective obstruction in five lambs that were not shunted. All survived. In three lambs we failed to

obtain complete obstruction, usually because the urachal ligation was incomplete. There was no significant difference between the body weight or the crown-to-rump length and kidney weight (right or left kidneys) between the low-pressure valve and high-pressure valve shunt groups (Table 1). There was a significant difference between the crown-to-rump length, body weight and kidney weights, between obstructed lambs and the normal controls ($p < 0.05$, $p < 0.01$). However, the bulk of the disparity in fetal weights and kidney weights between the obstructed lambs and the normal controls can be explained by the large increase in the bladder volumes and the presence of urinary ascites and/or unrinomas. The huge disparity between the bladder volumes in the three Group H lambs makes it impossible to make statistical comparisons between the bladder volumes in the three groups of obstructed lambs, given that one lamb in Group H with a high-pressure valve shunt had a huge dilated urachus containing approximately 1,150 ml of urine, which made it difficult to measure the exact bladder volume. This lamb was excluded from the calculation of bladder volume (Fig. 3a). The five non-shunted obstructed lambs (Group O) had urinary ascites and/or a dilated urachus (Fig. 3b). All the high-pressure valve shunt groups also had huge urinary ascites or a dilated urachus, but none of those in the low-pressure valve shunt group had either. The mean bladder volume is different in the three groups but if the lamb with a high-pressure shunt with the huge bladder volume is excluded as an outlier, then there are only two lambs in this group and statistical analysis for this group is impossible. However, in Table 1, the actual bladder volumes of all three lambs are shown.

Microscopically, the low-pressure valve shunt lamb kidneys had hydronephrosis or slightly dilated tubules but no dysplastic changes (Table 2). One lamb with a high-pressure valve shunt had 1,045 ml of retroperitoneal fluid and 1,150 ml of urine in the huge dilated urachus, and had histologically normal kidneys. One lamb with a high-pressure valve shunt without urinoma or urine ascites had renal dysplastic changes. The other high-pressure valve shunt lamb had dilated tubules and a reduced number of glomeruli but the proximal and distal tubules were well developed (Fig. 4).

Histologic examination of the bladders in the high-pressure valve shunted and no shunt obstruction lambs showed that the bladders from both groups had increased fibrosis in the submucosal and muscle layers compared with the bladders from the low-pressure valve lambs

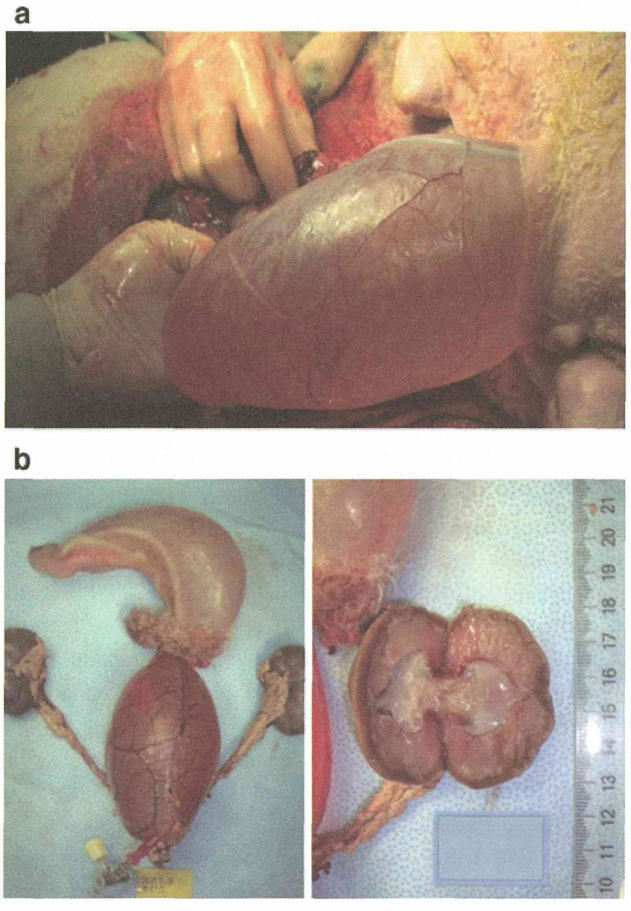

Fig. 3 **a** High-pressure valve shunt model with dilated urachus. **b** This non-shunted lamb had a dilated urachus with dysplastic kidneys

Table 1 Comparison of the body weight, crown-to-rump length, weight of right and left kidneys, bladder volume in low-pressure valve shunt (Group L), high-pressure valve shunt (Group H), obstruction (Group O) and control (Group C) groups

	Low valve shunt ($n = 4$)	High valve shunt ($n = 3$)	Obstruction ($n = 5$)	Control ($n = 4$)
Body weight (g)	3,407 ± 240	3,942 ± 1481*	3,984 ± 340*	2,377 ± 586
Crown-to-rump length (cm)	43 ± 3.7	44.6 ± 5.5	48 ± 2.1**	37.8 ± 4.3
Kidney weight (right) (g)	26 ± 4.3*	23.6 ± 7.1	37 ± 15**	7.2 ± 0.9
Kidney weight (left) (g)	20 ± 4.5	25.2 ± 4.4	35 ± 14**	8.8 ± 0.7
Bladder volume (ml)	116 ± 68	392 ± 656.7 (10, 15, 1,150 ml)	129 ± 100	24.5 ± 4.4

* $p < 0.05$ (Groups L, H, or O vs. Group C)

** $p < 0.01$ (Groups L, H, or O vs. Group C)

Table 2 Comparison of the urine ascites, dilated urachus, and histological findings of the kidney of low-pressure valve shunt, high-pressure valve shunt, and obstructed model

Group	Dilated urachus, urine ascites	Hydronephrosis	Dilated tubules	Reduced nephrons
Low valve shunt ($n = 4$)	0	3 (75 %)	1 (25 %)	0
High valve shunt ($n = 3$)	3 (100 %)	0	1 (33 %)	0
Obstruction ($n = 5$)	5 (100 %)	0	2 (40 %)	2 (40 %)

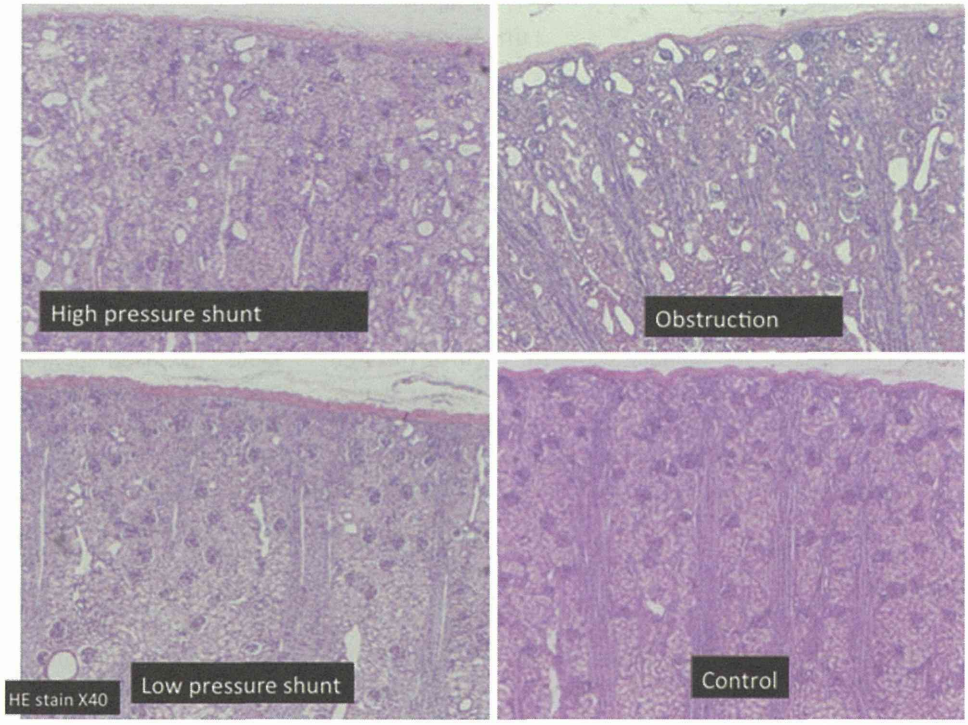

Fig. 4 Renal histology of high-pressure valve, low-pressure valve, obstructed lambs and controls. The high-pressure and obstructed lambs had dilated tubules but these were not seen in low-pressure valve shunt lambs. The histology of control lambs is similar to low-pressure valve shunt lambs

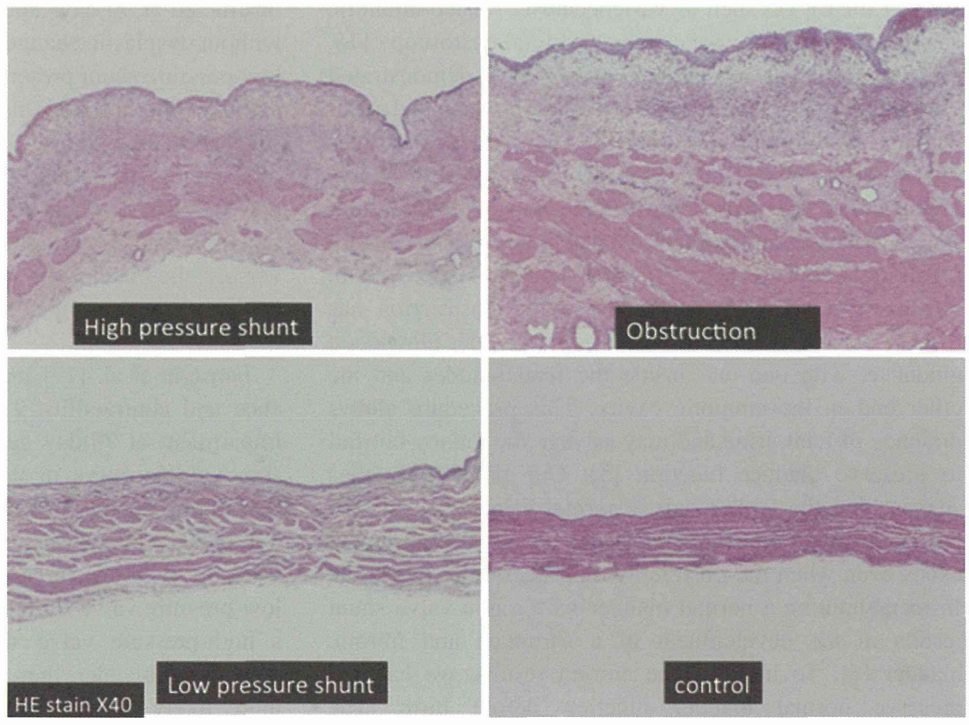

Fig. 5 Histology of the bladders in the four groups. The high-pressure valve shunted and no shunt obstruction lambs had increased fibrosis in the submucosal and muscle layers compared with low-pressure valve lambs. The bladder wall in control lambs did not have any fibrosis

Table 3 Comparison of the bladder thickness in each groups. After staining with hematoxylin and eosin stain, the distance from the submucosa to the outermost of muscle layer in the bladder wall was measured

Type of model	Bladder thickness (μm)
Control ($n = 4$)	338 ± 118
Obstruction ($n = 5$)	1,953 ± 941*
High valve shunt ($n = 3$)	1,479 ± 505**
Low valve shunt ($n = 4$)	1,018 ± 230**

Compared to controls * $p < 0.01$, ** $p > 0.05$

(Fig. 5). There is an impression that the bladder wall thickness in the bladders from low-pressure valve shunt lambs is less than that found in the high-pressure valve shunt and non-shunted lambs, but this failed to reach statistical significance. These bladders compare very favorably with the bladders of the normal control lambs. The mean thickness of the bladder walls for each of the four groups of lambs was 338 ± 118 (μm) in the control lambs, 1,953 ± 941 (μm) in the obstruction lambs, 1,479 ± 505 (μm) in the high-pressure shunt model, and 1,018 ± 230 (μm) in the low-pressure shunt model (Table 3).

Discussion

Fetal intervention for severe lower urinary tract obstruction (LUTO) is associated with high rates of perinatal mortality and postnatal renal impairment and bladder dysfunction [14]. Different therapeutic fetal interventions have been utilised for LUTO such as vesicocentesis, vesico-amniotic shunting, open fetal vesicostomy and fetal cystoscopy [15, 16]. Experimental and clinical studies have demonstrated that fetal urine retention causes severe bladder and renal damage and impairment [6, 7]. The bladder becomes dilated with a thick wall and progressive bilateral hydroureter and severe hydronephrosis develops. This presentation is associated with renal dysplasia and impairment of renal function [11, 12]. The most common approach in fetuses with an apparent bladder outlet obstruction has been to place a double pig-tailed catheter under ultrasound guidance, with one end inside the fetal bladder and the other end in the amniotic cavity. This procedure allows drainage of fetal urine and may salvage the kidney but fail to preserve bladder function [5]. Our previous studies suggest that the fetus needs a normal urination cycle for normal bladder development [5, 8, 9]. This requirement exists even when the obstruction is successfully bypassed. In fact, shunting a normal bladder with a non-valve shunt results in the development of a shrunken and fibrotic bladder [6]. To improve the current results, we have to preserve normal bladder function before birth. The suggestion from our research is to shunt the dilated bladder using a valved shunt tube which provides a "pop-off" mechanism in the developing bladder to avoid bladder fibrosis [8, 9]. We have previously shown in a similar fetal lamb model shunted 21 days after creating a bladder outlet obstruction at 60-day gestation, using the valve end of a ventriculo-peritoneal shunt (low pressure 15–54 mmH_2O valve shunt), that when the lambs were delivered at term (145 days), then the bladder volume (measured and compared to normal term fetuses), the bladder compliance and the histology of the bladder wall were almost normal [8, 9]. However, we have not defined the optimal pressure for a valved shunt that will preserve bladder function in the developing bladder.

In the current study, the lambs with a high-pressure V-P shunt tube (Group H: 95–150 mmH_2O) had outcomes similar to those in non-shunted obstructed lambs [5, 11]. All had a dilated urachus or urinary ascites. In non-shunted lambs, the histological appearances in the kidneys are more related to the presence of urinary ascites or urinoma, suggesting that these provide "pop-off" relief of the pressure in the renal tract, allowing preservation of renal development in at least one kidney. The lambs with a low-pressure valve shunt demonstrated hydronephrosis in 75 % of the lambs, but none of them had dilated tubules or reduced nephrons. These results suggest that a low pressure shunt is the appropriate shunt to use at 81 days gestation (60 + 21 days) to use to shunt a fetal lamb with a bladder outlet obstruction. The hydronephrosis observed is probably a reflection of the dilatation that occurred before the shunt was placed. In one of our previous studies, all lambs obstructed at 90 days and not shunted had hydronephrosis without dysplastic changes [11]. We have also shown that a low-pressure shunt preserved renal development [8, 9]. The autopsy findings and histological findings in this study suggest that the high-pressure valve shunts did not drain in fetal lambs and produced results similar to those seen in non-shunted obstructive uropathy lambs. The renal histology in these two groups of lambs is also similar. Conversely, the bladder and renal histology in the lambs with a low-pressure shunt is similar to that in normal controls of the same gestation.

Farrugia et al. [17] measured the fetal bladder compliance and contractility 9 days after creating urinary flow impairment at 75-day gestation in fetal lambs. The pressure–volume curve in the sham-operation group demonstrated that the normal resting bladder pressure seems to be 3–8 cmH_2O/ml. In the obstruction group the pressures were up to 10–20 cmH_2O, which is similar to that in our low-pressure valve shunt tube. In contrast, when we placed a high-pressure valve shunt (95–150 mmH_2O) into the developing bladder, then it did not drain well and behaved more likely our completely obstructed lambs' bladders.

Histological examination shows that the high-pressure shunt produces a bladder that is similar to that in non-shunted lambs. This would suggest that our low-pressure V-P shunt tube (15–54 mmH_2O) is a reasonable pressure to use for the fetal sheep bladder.

Pressure makes a difference in the developing bladder and kidney. Both the timing of shunting and the pressure of the shunting tube are critical to preserve normal renal development in utero and normal bladder function after birth.

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Conflict of interest The authors declare that they have no conflict of interest.

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■ 特集 胎児治療の最前線と今後の展望

出生前診断された横隔膜ヘルニアの胎児治療の適応と予後

臼井規朗*

はじめに

出生前診断された横隔膜ヘルニア（本症）に対する胎児治療は，当初胎児直視下手術として開始されたが，その後欧州の施設を中心にして，より低侵襲な経皮的胎児鏡下気管閉塞術（percutaneous fetoscopic endoluminal tracheal occlusion：FETO）へと発展してきた。現在，欧州およびカナダでは FETO を用いたランダム化比較試験（randomized controlled trial：RCT）が進行中で，これに北米や南米，オーストラリアの一部施設が参加を予定している。わが国では現在まで FETO による胎児治療は行われていないが，今まさに国内でも開始されようとしている。同時に，わが国では gentle ventilation の概念の普及により本症に対する出生後の治療技術が進歩し，治療成績が急速に改善しつつある。低侵襲になったとはいえ，母体および胎児にリスクを伴う本症の胎児治療については，そのリスクや予後と最新の出生後の治療成績とを比較して，慎重に適応を決める必要がある。

本稿では，本症の胎児治療の歴史を振り返りながら，文献的に報告された胎児治療の適応と予後について概説する。さらに，わが国で行われた多施設共同研究および全国調査の結果から，わが国の最新の出生後の治療成績にもとづいて，本症の胎児治療における適応と問題点について考察する。

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Ⅰ．欧米における胎児治療の歴史

本症に対する最初の胎児治療は，胎児に対して直視下に出生後と同様の横隔膜ヘルニア修復術を行うというものであった。しかし，重症の肺低形成を伴うような肝脱出例では，臍静脈の屈曲のため成功せず，この方法は以後行われなくなった[1,2]。以前より，気道内の陽圧が肺の発育を促進することが知られていたが，それを実験的に本症の治療に応用したのは Wilson ら[3]のグループであった。それ以後，数々の動物実験によって本症に対する胎児気管閉塞術の有用性が示されてきた。

胎児気管閉塞術を初めて臨床応用したのは Harrison らのグループであった[1,2]。気管閉塞の方法は，気管内腔に発泡体の栓をすることから始まり，気管に直接クリップをかける方法，その後さらに離脱型バルーンの留置へと変遷していった。またその手術手技も，直視下手術から子宮を露出した状態での胎児鏡下手術へと移行していった。初期の報告では，左側の本症単独例で，肝脱出かつ肺断面積頭周囲長比（lung area to head circumference ratio：LHR）＜1.4 の症例を適応とし，非胎児治療例が 13 例中 5 例生存（38%）のところ，直視下手術では 13 例中 2 例（15%），胎児鏡下手術では 8 例中 6 例（75%）が生存したと報告した[4]。同じころ，Adzick ら[5]のグループは，左側本症単独例で肝脱出かつ LHR＜1.0 の症例を適応として，直視下に気管にクリップをかける方法で治療を行い，15 例中 5 例（33%）が生存したと報告した。しかし，生存例 5 例中 4 例には中枢神経障害が残存した[5]。これらの結果にもとづき，北米

で本症の胎児治療としては初めて RCT が行われた[6]。左側本症単独例で，肝脱出かつ LHR＜1.4 の症例を適応として，母体の開腹後胎児鏡下に離脱型バルーンを留置する手技であった。気道閉塞の解除は，分娩時の EXIT（*ex utero* intrapartum therapy）によって行われた。13 例に出生後のみの治療が，11 例に胎児治療が行われたが，それぞれの生存率は 77%，73%と胎児治療の有効性は証明されず，RCT は途中で中止された[6]。

一方，欧州では Deprest ら[2]を中心にして，より低侵襲に治療を行うことを目的に，母体を開腹せず 3.3 mm の細径胎児鏡 1 本で経皮的に治療が完遂できる FETO が考案された。当初母体の全身麻酔下に行われたが，すぐに局所・部分麻酔で行うようになり，手技に要する時間も非常に短縮された。在胎 34 週ごろに再度胎児鏡などを用いて気道閉塞の解除を行うことを原則とし，約 70%の症例で分娩前の気道閉塞解除に成功している[2]。

胎児治療の適応については，当初 27 週前後の LHR をそのまま用いて，LHR＜1.0 かつ肝脱出のある左側・右側症例としていた。しかし，LHR の正常値が在胎期間の経過とともに 5〜6 倍に増加する[7]ことが明らかになると，在胎期間ごとの正常児の平均 LHR を標準値とし，これに対する患児の LHR の比を計算して observed to expected LHR（O/E LHR）として用いるようになった。肺の断面積の測定法においても，前後径と左右径を計測する原法や，長径と短径を計測する longest 法より，肺の辺縁をトレースして断面積を測定する方法がもっとも再現性が高いとされた[7]。現在，日本人の標準値は利用できないが，欧州の標準値にもとづく O/E LHR の計算式については論文上で公開されている[8]。

O/E LHR は，在胎期間相当の正常児の平均値によって標準化するため，個々の胎児の発育の程度によっては誤差が大きくなってしまうという問題点がある。しかし，在胎期間を通じて正常域が一定であり，健側肺の断面積を肺の辺縁のトレースによって計測する点など，わが国で LHR と同様に比較的広く用いられている肺胸郭断面積比（L/T 比）との共通点も多い。

II．欧米における胎児治療の適応と治療成績

Deprest ら[9]は，LHR＜1.0 に相当する O/E LHR を示す肝脱出のある左側・右側症例を適応として，本症の胎児 210 例に対して 2001〜2008 年に胎児治療を行い，成績を以下のように報告した。FETO が施行された在胎週数の中央値は 27.1 週で，210 例のうち初期の例を除く 96%の症例が局所・部分麻酔下に施行され，97%の症例でバルーン留置に成功した。母体の合併症としてもっとも問題となる前期破水の発症率は，FETO 施行後 3 週間以内では 17%にとどまり，34 週以前に生じた前期破水の割合も 25%であった。北米の RCT で問題となった早産については，34 週以前の分娩が 31%にとどまった。97%の胎児が生存出生し，48%の患児が生存退院した。左側例では生存率は 24%から 49%に，右側例では 0%から 35%に改善した。また児の合併症として気管軟化症を引き起こす tracheomegaly についても，努力性呼気時の金属性咳嗽のみで，次第に軽減されていくとした[2]。ほぼ同様の適応で FETO が行われたほかの施設についても，ドイツ（生存率 75%；6/8 例）とブラジルの 2 つの施設（生存率 58%；7/12，生存率 42%；5/12）から報告がなされている。

しかし，この時期は gentle ventilation，あるいは permissive hypercapnia などの概念の普及により，出生後の治療成績も著しく向上した時期と重なるため，単純に前時代の症例の予後と比較するだけで胎児治療の有効性を判断するわけにはいかない。

III．胎児治療における RCT としての TOTAL trial

本症に対する胎児治療が格段に低侵襲となり，その有効性が報告されるようになってきた一方で，同時に出生後の治療成績も向上してきた。このような環境のもとでは，RCT を行う以外に本症に対する胎児治療の有効性を証明する方法はない。そこで 2008 年に欧州とカナダの施設が共同で FETO を用いた Tracheal Occulusion To Accelerate Lung Growth trial（TOTAL trial）とよばれる

RCT を開始した。今後，北米や南米，オーストラリアの一部施設もこの研究への共同参加を予定している。そのプロトコールは次稿に詳述されているため省略するが，大きく分けて 2 つの臨床試験で構成されている。すなわち，プライマリアウトカムを患児の生命予後においた重症例に対する臨床試験と，患児の出生後の肺機能においた中等症例に対する臨床試験である。各臨床試験への適応は単胎，本症単独，左側例に加え，肺低形成の重症度によって判定されるが，肺低形成の重症度判定において O/E LHR がもっとも重視されている点が特徴といえる。例えば，重症例の臨床試験では O/E LHR<25%のみで適応とされ，肝脱出の有無は問われない。

Ⅳ. わが国における胎児治療の適応症例数と問題点

わが国では，2012 年 10 月現在まで FETO による胎児治療は行われていないが，今まさに国内でも FETO が開始されようとしている。FETO の導入期にあたるわが国では，参加要件を満たす限定された施設が TOTAL trial に参加して，統一プロトコールのもとに開始することが望ましい。当面は単一の施設へ症例を集中するため，胎児治療の候補症例を全国から拾い上げて集約化するシステムの構築が必要となる。

その際，症例の発見時期が重要となる。例えば TOTAL trial の重症例臨床試験では，在胎 29 週 5 日までに適応を判断する必要がある。しかし，実際にわが国のどこかで候補症例が発見された場合，臨床試験の情報提供を受けて希望した母体が中央施設に紹介され，中央施設での精査と臨床試験参加のインフォームドコンセント取得を経て適応が最終決定される。各段階に要する時間を想定すれば，遅くとも在胎 27 週ごろまでには発見されていることが望ましい。

わが国における胎児治療の適応を考える場合，2008 年に国内 5 施設により行われた多施設共同研究が一つの参考になる。本研究は出生前診断された本症単独例 117 例（2002～2007 年）についての後方視的コホート観察研究として行われた[10]。生後 90 日の生存率は 79%（92/117 例）であり，酸素，人工呼吸，経管栄養，肺血管拡張薬投与などの在宅治療を必要とせずに退院できた「合併症なき退院」の割合は 63%（74/117 例）であった[10]。すなわち，25 例が生後 90 日以内に死亡し，以降に死亡した症例と，合併症を有したまま退院した症例を加えると，計 43 例は「合併症なき退院」ができなかった。しかしこのうち，在胎 27 週以前に診断されていた症例は半数以下の 17 例にとどまる。胎児治療の適応範囲を合併症を有したまま退院する症例にまで広げるとしても，わが国の出生前診断時期が諸外国に比べて遅いことが，胎児治療推進の妨げとなっていることがわかる。

同様のことは，2011 年にわが国で実施された本症の全国調査[11]からもいえる。全国調査には国内の 72 施設が参加し，本症の 614 例（2006～2010 年）が集計された。日本小児外科学会が行っている 5 年ごとの新生児外科統計における年間症例数（約 180 例）から類推すると，本調査ではわが国の半数を超える症例が調査されたものと推測された。過去 5 年間の 614 例中，本症単独例は 520 例（85%）であり，そのうち出生前診断された症例は 364 例（70%）であった。これらの症例の出生前診断週数の分布を示した（**図**）。63 例（黒）が生後 90 日以内に死亡し，45 例（灰色）が生後 90 日以降に死亡したか，合併症を有したまま退院した。在胎 27 週以前に発見された症例（点線より早期）が FETO の適応になりうると仮定すれば，死亡例のみを適応とした場合 32 例となり，合併症を有して退院した例を含めても 54 例であった。国内の約半数例が調査できたと仮定すると，わが国における胎児治療の候補症例数は，死亡例を適応とした場合年間 14 例，合併症を有して退院する例を含めても年間 22 例と概算される。胎児治療の候補症例のうち全例が中央施設への搬送や胎児治療を希望するとは考え難く，さらに RCT の振り分けにより約半数には FETO が実施されないことも考えれば，わが国で実際に施行される FETO の症例数は自ずと限定されてくる。今後，胎児スクリーニングを整備して，より早期に本症を発見しない限り，症例数の増加は見込めないと思われる。

しかし一方で，診断時期が早ければ早いほど適応症例が増加するかといえば，若干疑問が残る。

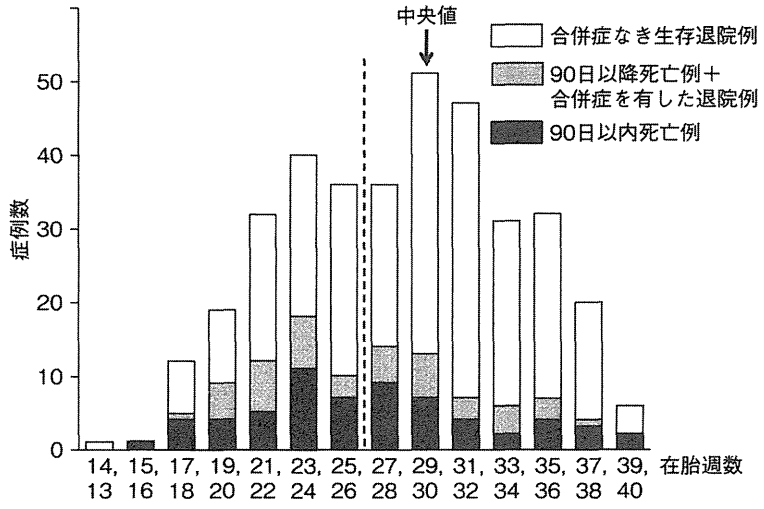

図　新生児横隔膜ヘルニア全国調査（2011年）における本症単独症例の出生前診断時期と予後との関係（臼井ら[11]，2012）

わが国では，在胎22週未満の妊婦には，母体理由による人工妊娠中絶が法的に認められている。出生後治療だけで救命可能と見込まれる症例であっても，ときに人工妊娠中絶が選択されている現状を考えれば，在胎22週以前の早期診断例の増加は，かえって本症の胎児治療適応例を減少させる可能性もはらんでいる。

V．わが国における胎児治療の適応基準と問題点

わが国のFETO導入期に，TOTAL trialに参加する施設が統一プロトコールのもとでFETOを実施する際には，胎児治療の適応基準で戸惑うことはなかろう。しかし，本来リスクを伴う治療の適応の決定は，各施設や地域，あるいは国におけるその時代の治療実態や治療成績などと治療効果を比較したうえで行うべきであろう。TOTAL trialのような国際的な多施設共同研究の終了後は，治療実態や治療成績の異なる地域や国で，共通の適応基準を堅持する意味合いは乏しい。したがって，将来的にはわが国の治療実態に即した，わが国独自の適応基準が必要になると思われる。

前述の共同研究の結果，Kitanoら[12]は，本症左側例の胃の位置に関する新たな分類を提唱し，重症度予測における胃の位置の重要性を述べている。肝脱出の有無と胃の位置の組合せから，肝脱出を伴わないGroup I，肝脱出を伴い胃の位置がGrade 0～2のGroup II，肝脱出を伴い胃の位置がGrade 3のGroup IIIに分けると，「合併症なき退院」が可能であった割合はそれぞれ87%，47%，10%であった[12]。このGroup IIIをもって胎児治療の適応とするという意見もある[13]。いずれにしても手技の習熟を必要とする計測を行うことなく，高リスク症例を簡便に判定できる意義は大きく，FETOの候補症例を拾い上げるための一次スクリーニングとしても応用可能であろう。

また同研究では，肝脱出とL/T比を組合わせて出生前診断例のリスクを層別化することも試みられている[14]（**表**）。もっとも重症なC群では，90日生存率が35%，「合併症なき退院」の割合が5%ときわめて予後不良であったのみならず，さまざまなパラメータが治療の困難さを示していた[14]。わが国でFETOが本症の治療法の選択肢の一つとして確立されれば，この群のような重症度をもつ症例が胎児治療の適応になるのかもしれない。

TOTAL trialにみられるように，肺低形成の国際的な評価法はO/E LHRが一般的となりつつある。O/E LHRはL/T比と指標としての類似性が強いため，両者に強い一次相関があると推測される。今後両者を比較して相互に換算する方法を，早急に検討する必要がある。

表 先天性横隔膜ヘルニアの出生前診断例におけるリスク層別化分類別にみた症例の重症度比較

リスク分類	Group A	Group B	Group C	
判定基準[注1]	肝脱出（−）かつ L/T 比≧0.08	肝脱出（−）かつ L/T 比<0.08, 肝脱出（+）かつ L/T 比≧0.08	肝脱出（+）かつ L/T 比<0.08	
症例数	n=48	n=35	n=20	*p*
動脈管左右優位（%）[注2]	39.1（n=46）	36.4（n=33）	0.0（n=18）	0.007
動脈管右左優位（%）[注2]	37.0（n=46）	51.5（n=33）	72.2（n=18）	0.036
PGE_1 の使用率（%）	14.6	40.0	70.0	<0.001
NO 吸入療法の施行率（%）	70.8	94.3	95.0	0.005
ECMO の施行率（%）	2.1	14.3	40.0	<0.001
NO 吸入療法期間（日）¶	8（5〜12）*	11（7〜19）†	34（22〜40）‡	<0.001
人工呼吸期間（日）¶	14（9〜28）*	30（21〜48）†	545（30〜747）‡	<0.001
酸素投与期間（日）¶	23（15〜38）*	43（37〜73）†	555（529〜748）‡	<0.001
入院期間（日）¶	48（39〜69）*	73（56〜108）	162（95〜545）‡	<0.001
手術不能例（%）	0.0	11.4	35.0	<0.001
75%以上の横隔膜欠損例（%）	17.8（n=45）*	81.5（n=27）*	100.0（n=11）*	<0.001
パッチ閉鎖手術例（%）	20.8（n=48）	71.0（n=31）	92.3（n=13）	<0.001
90 日生存率（%）	100.0	80.0	35.0	<0.001
生存退院率（%）	100.0	74.3	20.0	<0.001
合併症なき退院率（%）	95.8	60.0	5.0	<0.001

*p<0.05 A vs B, †p<0.05 B vs C, ‡<0.05 C vs A, ¶中央値（四分位範囲）

注1) 出生前の画像診断で胸郭の 1/3 以上肝が脱出しているものを肝脱出（+）とした。胸郭の 1/3 に満たない脱出や，手術時に初めて肝脱出が判明した症例は肝脱出（−）とした。

注2) 出生後 24 時間以内に判定した。

（Usui ら[14]，2011 より引用，一部改変）

全国調査から明らかとなったもう一つの問題点は，出生前診断された 442 例中 LHR が計測されていた症例は 57%（240/422 例），L/T 比が計測されていた症例が 55%（231/422 例）と 6 割にも満たなかった[11]点である。Kitano の分類[12]など，どの施設でも簡便にリスクを判別できる方法を含め，肺低形成の正しい評価方法を広く啓蒙する必要がある。

最後に，わが国では本症に対して FETO とは全くコンセプトの異なる内科的胎児治療として，低形成に陥った本症胎児の左心室を母体酸素投与によって育成するという発想に立つ新しい胎児治療の試みも開始されつつある[15]。今後の研究成果に期待したい。

おわりに

いよいよわが国でも，TOTAL trial に参加した形で FETO が開始されようとしている。もしこの RCT で本症に対する胎児治療の有効性が明らかとなれば，わが国でも本格的に FETO が本症の治療手段の選択肢の一つとなる。来たるべき日に備え，わが国の多数の施設が互いに協力しあい，本症の胎児治療を推進できるシステムを構築すべきであると思われた。

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■ 特集 胎児治療の最前線と今後の展望

実験的胎児治療：新たな膀胱羊水腔シャントチューブの開発

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はじめに

胎児治療は経母胎的に子宮内の胎児に認められた疾患に対して行う治療で，出生後の治療では救命できない疾患をもった患児が治療の対象であった。小児外科の歴史では，Harrison ら[1]が子宮を切開して行う横隔膜ヘルニア手術の成功を 1982 年に報告後，胎児の子宮内での病態生理がより明らかとなり，その治療の適応が拡大した。しかし，近年の周産期医学の進歩により，出生後の治療で救命可能な疾患が増え，再び治療の適応は絞られてきた。筆者らは胎児治療のなかで比較的診断が容易で，治療侵襲の少ない閉塞性尿路疾患に注目し研究を行ってきた。また，本疾患の長期予後が期待されたほど良好でなく，出生後の排尿障害や腎機能障害が報告され[2]，これらの原因を明らかにするため，大動物を用いた研究を行ってきた。本稿では今までの実験的胎児治療の経過と，これまでのシャントチューブの開発の経緯を述べる。

Ⅰ．実験的胎児治療の変遷

1．ヒツジを用いた胎仔尿路閉塞モデルの歴史

ヒツジを用いた実験的尿路閉塞モデルは今から 40 年前の 1972 年ごろから行われ[3]，胎生 60 日のヒツジ胎仔では尿膜管と尿道の両者を閉塞しなければ尿路閉塞モデルは作製できないことが明らかとなり，その後 1980 年代に入り Golbus, Glick, Harrison ら[4,5]による種々の実験モデルが考案された。筆者らの実験開始時には，メスの尿路閉塞モデルの良好な成績の報告がなく，われわれは胎仔の膀胱頸部を結紮するモデルを作製した（図 1）。今までの実験結果から，胎生 60 日の尿路閉塞では，145 日の満期には腎に嚢胞性変化を伴った多嚢胞性異形成腎（multicystic dysplastic kidney：MCDK）様所見が認められ，筆者らはこの拡張が近位尿細管であることを報告した[6]。その後，閉塞性尿路障害に対する胎児手術の適応と治療のタイミングをヒツジを用いた大動物から考察し[7]，また尿路閉塞の胎生時期による相違で異なる腎病変が認められ，胎生早期の尿路閉塞では腎の線維化が高度で羊水過小を呈する Potter sequense モデルがヒツジを用いて初めて作製で

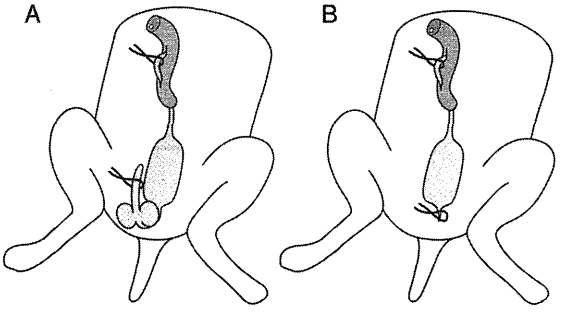

図 1 ヒツジ胎仔尿路閉塞モデル
A．尿膜管と陰茎尿道を結紮したオスの尿路閉塞モデル。
B．尿膜管と膀胱頸部を結紮したメスの尿路閉塞モデル。

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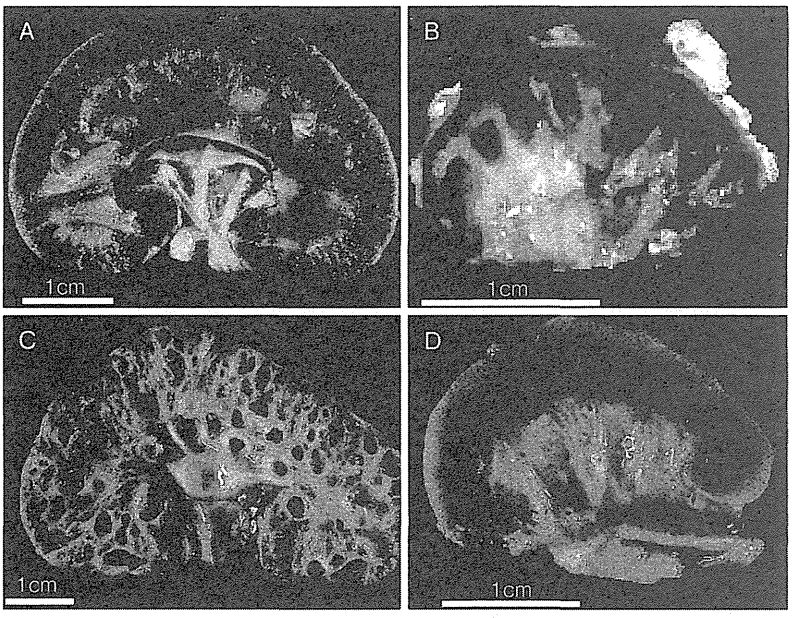

図 2 胎生 50 日，60 日で尿路閉塞作製後の腎のマクロ所見

A. コントロール，**B**. 50 日閉塞モデル，**C**. 60 日閉塞モデル，**D**. 60 日閉塞モデル。
50 日の閉塞ではネフロンの形成がほとんど認められず羊水過小を呈した。腎は萎縮し小さい。下段の 60 日では大きな嚢胞を認める場合と小さな嚢胞を認めるモデルの両者が表現系として認められた。

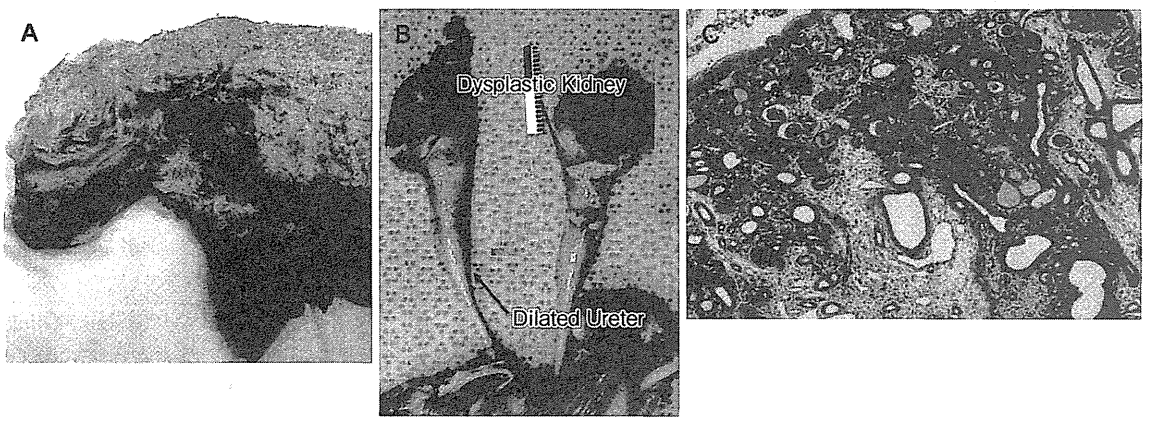

図 3 50 日尿路閉塞で羊水過少症を伴ったモデル

A. 四肢の屈曲，つぶれた鼻など Potter 症候群に類似したヒツジを示す。**B**. 摘出した尿路を示すが，小さな萎縮した腎臓と拡張した尿管を認めた。**C**. 腎の組織所見を示す。嚢胞形成は少なく，線維化の多い萎縮腎を呈していた。

きた[8,9]（図 2，3）。

2．ヒツジを用いた膀胱-羊水腔シャントの効果

胎生 60 日に尿路閉塞を作製し，その後，20～23 日，24～27 日，28～31 日経過後の腎の変化を観察した。嚢胞形態は大小さまざまであったが，尿路閉塞後 3 週間で，すでに満期で認めたのと同様の MCDK 様所見を呈し，V-A シャント時期を閉塞後 3 週とした[7]。22 匹に尿路閉塞後 3 週間で V-A シャントを作製し，13 匹が生存した。そのうち 6 匹（55%）は正常腎組織構造を呈し，2 匹の腎はサイズが小さく，腎の萎縮を認め，顕微鏡的にもネフロン数の少ない低形成腎であった。また，3 匹にシャントの効果が乏しく，MCDK 様所見の残存を認めた[10]（図 4）。また，この時点で V-A シャントを施行し，たとえ腎を温存できたとしても膀胱壁の線維化が著明で，膀胱機能が廃絶することが明らかとなり，拡張膀胱に急激なシャントを行うことが膀胱壁の線維化を増強させ，膀胱