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ORIGINAL ARTICLE

A new ovine model of spine and chest wall deformity at birth with alteration of respiratory system mechanics and lung development: a feasibility study

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Abstract

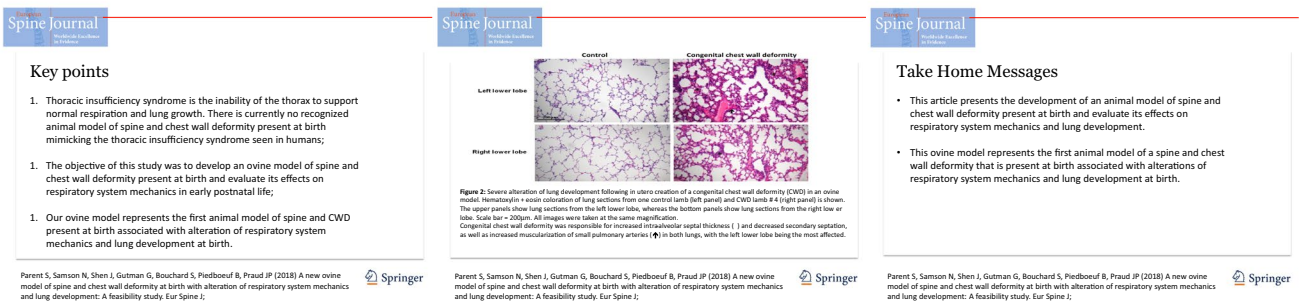
Purpose To develop an animal model of spine and chest wall deformity (CWD) at birth and to evaluate its effects on respiratory system mechanics and lung development.

Methods A spine and CWD was created in utero between 70 and 75 days of gestation in six ovine fetuses by resection of the seventh and eighth left ribs. Two days after birth, respiratory system mechanics was assessed in anesthetized lambs using the flexiVent apparatus, followed by postmortem measurement of lung mechanics as well as histological lung analysis.

Results A range of severity of CWD was found (Cobb angle from 0° to 48°) with a mean decrease in compliance of 47% and in inspiratory capacity of 39% compared to control lambs. Proof-of-concept histological analysis in one lamb showed marked lung hypoplasia.

Conclusion Our ovine model represents a pilot proof-of-concept study evaluating the impact of a spine and CWD present at birth on lung respiratory mechanics and development. This study lays down the groundwork for future studies evaluating the impact of these deformities on lung development and potential treatments.

Graphical abstract These slides can be retrieved under Electronic Supplementary Material.



Keywords Chest wall deformity · Fetal surgery · Respiratory function · Lamb · Animal model

Introduction

Spine and chest wall deformities (CWD) occur when there is anomalous skeletal development and/or formation of the thoracic cavity. This condition can affect normal thoracic growth and function as well as lung development. Moreover, these deformities can present either immediately at birth

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or develop during the postnatal period. Thoracic function depends on a stable diaphragm and on active rib motion, which in turn depends on the presence of separate ribs, intercostal muscles and symmetry of the thorax. Consequently, congenital malformations affecting thoracic volume or function may result in thoracic insufficiency syndrome [1], which is the inability of the thorax to support normal respiration and/or lung growth. Furthermore, lung growth is limited to the anatomic boundaries of the thorax, so any spine or rib cage malformation that reduces the thoracic volume early in life may adversely affect the size of the lungs at skeletal maturity [2]. A recent study has hence highlighted the halving of the vital capacity observed in adults with severe congenital scoliosis following spinal fusion between 3 and 7.6 years of age [3].

To our knowledge, there is currently no recognized animal model of spine and CWD mimicking the thoracic insufficiency syndrome seen in humans. Congenital human CWD represents a disease continuum that starts early during fetal life and progresses thereafter. Therefore, the detrimental impact of the thoracic deformity on lung development is initiated in utero and presents clinically at birth.

Thus, the main objective of this feasibility study was to develop an animal model of chest wall and spine deformity created in utero and to evaluate their effects on respiratory mechanics at birth.

Materials and methods

Animals

Experiments were conducted in six full-term lambs with spine and CWD born from four ewes and three full-term control lambs, aged 3 days. The ethics committee for animal care and experimentation of the Université de Sherbrooke approved the study.

In utero creation of the chest wall deformity

Fetal surgery was performed at 70–75 days of gestation (normal term of 147 days) under general anesthesia (isoflurane 2%; Baxter Corporation, Mississauga, ON, Canada). Under sterile conditions, a longitudinal laparotomy was performed exposing the uterine horns, followed by a hysterotomy. The lower limbs, abdomen and thorax of the fetal lamb were delivered, and the seventh and eighth left ribs were resected. Following fetal skin closure, each lamb was returned to the uterus and the amniotic fluid was replaced with warm Ringer's lactate containing G penicillin (Vétoquinol N-A Inc, Lavaltrie, QC, Canada). The membranes and uterus were closed followed by the abdominal wall. Once awake, ewes were returned to their pen and allowed to pursue the remainder of their gestation in our animal quarters until they gave birth spontaneously to their offspring.

Characterization of the ovine model of congenital chest wall deformity at birth

Vital signs, including respiratory rate (RR), heart rate (HR) and oxygen hemoglobin saturation (SpO_2) were formally assessed at 30 min after birth and then every 8 h until 2 days after birth. Lambs were kept with their mother from whom they fed ad libitum. At 2 days of life, vital sign measurement was complemented by chest X-ray to assess the degree of CWD according to the Cobb angle of the main curve. Cobb angle up to 30° was considered mild, whereas Cobb angle of 45° or more was considered severe. In addition, respiratory system mechanics was measured as follows to assess the impact of CWD on respiration.

In vivo assessment of respiratory function

Respiratory system mechanics was measured in anesthetized (IV propofol, 6 mg/kg/h; Baxter Corporation, Mississauga, ON, Canada), curarized (rocuronium, 0.1 mg/kg; Hospira, Saint-Laurent, QC, Canada) and ventilated lambs (tidal volume 8 ml/kg, respiratory rate 40/min, PEEP 2 cmH_2O, FiO_2 21%) using the flexiVent apparatus (SCIREQ Scientific Respiratory Equipment Inc., Montreal, QC, Canada). The respiratory system of the lamb was first inflated with air up to a pressure of 40 cmH_2O for 1 min and the inspiratory capacity computed. Then, the quasi-static compliance of the respiratory system was measured as follows: A standardized deflation pressure–volume curve was performed by computer-driven step changes of the pressure down to 20, 15, 10, 5 and 0 cmH_2O, the lung volume being computed after 30 s at each pressure. The Salazar–Knowles equation was used to account for the difference in lung/thorax size and known curvilinearity of the pressure–volume relationship [4].

Postmortem measurement of lung function

The tracheobronchial tree, lungs and mediastinum were resected en bloc to take postmortem measurements. Both the inspiratory capacity and lung compliance were measured with the flexiVent as described above, first for both lungs and then for each lung individually to assess whether consequences of CWD were different for the right and left lungs.

As a proof of principle, a histomorphological analysis of the lung was further performed in one lamb with a severe decrease in lung volume and compared to a control lamb. Following postmortem measurement of lung function, both right and left lungs were perfused with 10% formaldehyde under a pressure of 20 cmH_2O for 1 h and then immerged in 10% formaldehyde at 4 °C for 24 h. Following embedment

in paraffin, lung sections randomly sampled from both upper and lower lobes were cut in 4–5-µm-thick slices and stained with hematoxylin and eosin [5].

The present study was primarily designed to test the feasibility of developing a unique newborn model with altered respiratory function secondary to a CWD created surgically in utero. No postnatal follow-up was performed beyond the first 2 days of life at this stage.

Statistical analysis

Descriptive statistics included group means and 95% confidence intervals, as well as differences between groups. Given the low number of lambs in each group in this feasibility study, no statistical test could be run to look for differences between groups.

Results

Ovine model of congenital chest wall deformity

All lambs were born at term; a mild or severe CWD was observed in three and two lambs, respectively (Fig. 1). Mild deformities were characterized by a left thoracic curve and severe deformities by a left thoracolumbar curve. One lamb with a severe CWD (Cobb angle = 63°) was stillborn and could only be assessed by postmortem chest X-ray and respiratory mechanics measurement. The second lamb suffering from severe CWD (Cobb angle = 48°) rapidly developed a respiratory distress and was anesthetized at 30 min of life to undergo measurement of respiratory system mechanics, to be euthanized immediately thereafter. The other four lambs with CWD were alive with normal vital signs at 2 days of life (Table 1). None of the operated lambs developed or grew new ribs between the antenatal surgery and birth.

Measurement of respiratory system mechanics

In vivo assessment of respiratory system mechanics revealed a mean decrease in respiratory system compliance of 47% ((3.2 − 1.7)/3.2) (Mean control − mean CWD/mean control) and inspiratory capacity of 39% ((44.5 − 39)/44.5) in the deformity lambs compared to controls (Table 2). Similar results were obtained postmortem with, respectively, a mean decrease of 60% ((2 − 0.8)/2.0) for both lungs in respiratory system compliance (40% ((0.5 − 0.3)/0.5) left lung and 45% ((1.1 − 0.6)/1.1) right lung) and 56% ((57 − 25)/57) for both lungs in inspiratory capacity (52% ((16.5 − 8)/16.5) left lung and 52% ((33 − 16)/33) right lung) (Table 2).

In addition, the histomorphological analysis of one lamb with severe CWD showed a severely impaired alveolarization in both lungs, especially for the left lung, which was predominantly at a saccular stage of development (Fig. 2).

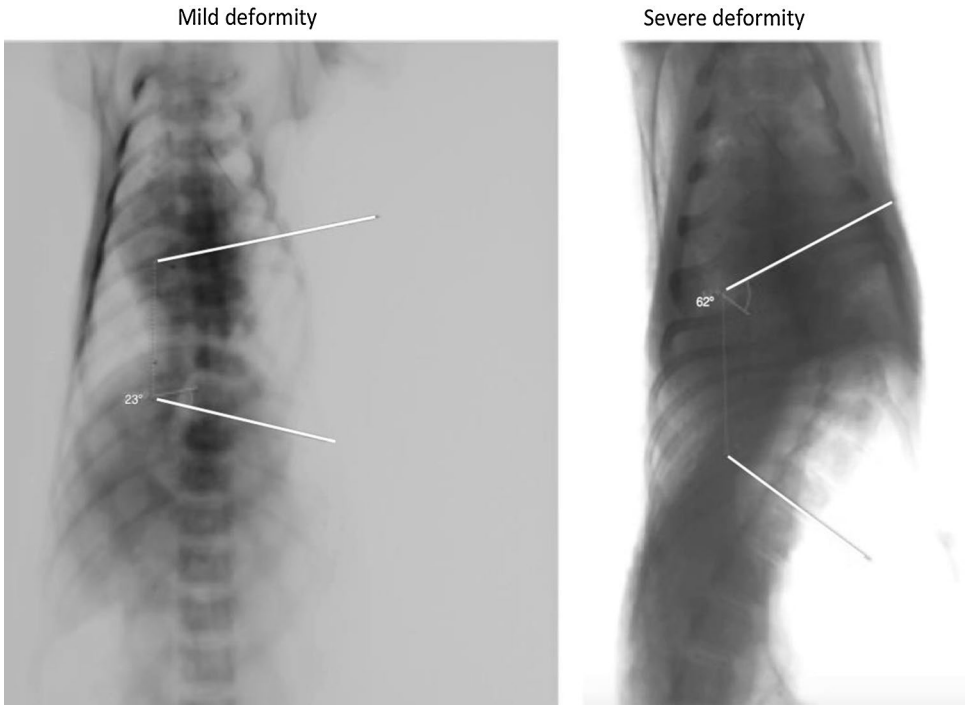

Fig. 1 Thoracoabdominal posteroanterior X-ray showing an ovine model with a mild to moderate: 23° (left panel, lamb #5) and severe: 63° (right panel, deceased lamb #3) congenital spinal deformity

Table 1 Vital signs of each lamb at birth

	Cobb angle (°)	Sex	Weight (kg)	RR (min^{-1})	HR (min^{-1})	SpO_2 (%)
Control 1	0	M	3.6	60	175	99
Control 2	0	M	4.1	50	180	98
Control 3	0	M	4.0	80	191	98
CWD 1	13	M	3.7	60	175	96
CWD 2	8	F	4.2	70	175	97
CWD 3 (stillborn)	63	M	3.5	–	–	–
CWD 4 (RD at birth)	48	F	4.2	80	197	86
CWD 5	0	F	2.1	100	222	98
CWD 6	0	F	3.0	70	222	99

CWD: lambs with in utero congenital chest wall deformity; M: male lamb; F: female lamb; RD: respiratory distress; RR: respiratory rate; HR: heart rate; SpO_2: oxygen hemoglobin saturation

Table 2 Measurements of respiratory mechanics in vivo and postmortem

	In vivo measurements		Postmortem measurements		
	IC (ml/kg)	Respiratory system compliance (ml/cmH_2O/kg)	IC both lungs–left lung–right lung (ml/kg)	Ratio left lung IC/right lung IC	Compliance both lungs–left lung–Right LUNG (ml/cmH_2O/kg)
Control 1	47	3.6	62.5–21.5–36	0.6	2.4–0.7–1.3
Control 2	48.5	3.7	64.5–17–38.5	0.4	2.0–0.6–1.3
Control 3	38	2.4	44.5–11–24	0.5	1.6–0.3–0.8
Mean	44.5	3.2	57–16.5–33	0.5	2–0.5–1.1–3)–(0.02–1.1)–(0.4–1.9)
Confidence interval	30.5–58.5	1.4–5.0	(30–84.5)–(3.4–29.5)–(13.6–52.1)	0.3–0.8	
CWD 1	35	2.3			
CWD 2	20.5	1.4			
CWD 3 (stillborn)			10.5–6–8.5	0.7	0.5–0.2–0.4
CWD 4 (at birth)	12.5	0.7	12.5–4.5–9	0.5	0.5–0.1–0.4
CWD 5	28.5	1.5	26–7.5–16	0.5	0.7–0.5–0.5
CWD 6	40	2.5	50–14.5–30	0.5	1.4–0.5- 1.1
Mean	27	1.7	25–8–16	0.55	0.8–0.3–0.6
Confidence interval	13.5–41	0.8–2.6	(−4.2 to 43.7)–(1.1–15.2)–(−0.1 to 31.8)	0.4–0.7	(0.1–1.5)–(0–0.7)–(0.1–1.1)
Difference of means	17	1.6	32.5–8.5–17	0.1	1.2–0.2–0.5

CWD, lambs with congenital chest wall deformity; in vivo measurements were taken in anesthetized and paralyzed lambs within the first 2 days of life; postmortem measurements were taken after euthanasia on ex vivo, isolated tracheobronchial tree + lungs; IC, inspiratory capacity (ml/kg); compliance (ml/cmH_2O/kg)

Discussion

The present feasibility study reports a unique ovine model of spine and chest wall deformity present at birth. The model allowed us to evaluate the effects of these deformities on lung respiratory function and structure in the immediate postnatal period.

Uniqueness of our ovine model of spine and chest wall deformity

Mehta et al. [6] previously proposed a model of thoracic growth disturbances created by either tethering the transverse processes or ribs unilaterally or bilaterally in 5-week-old rabbits. Their results showed increasing spinal curvature

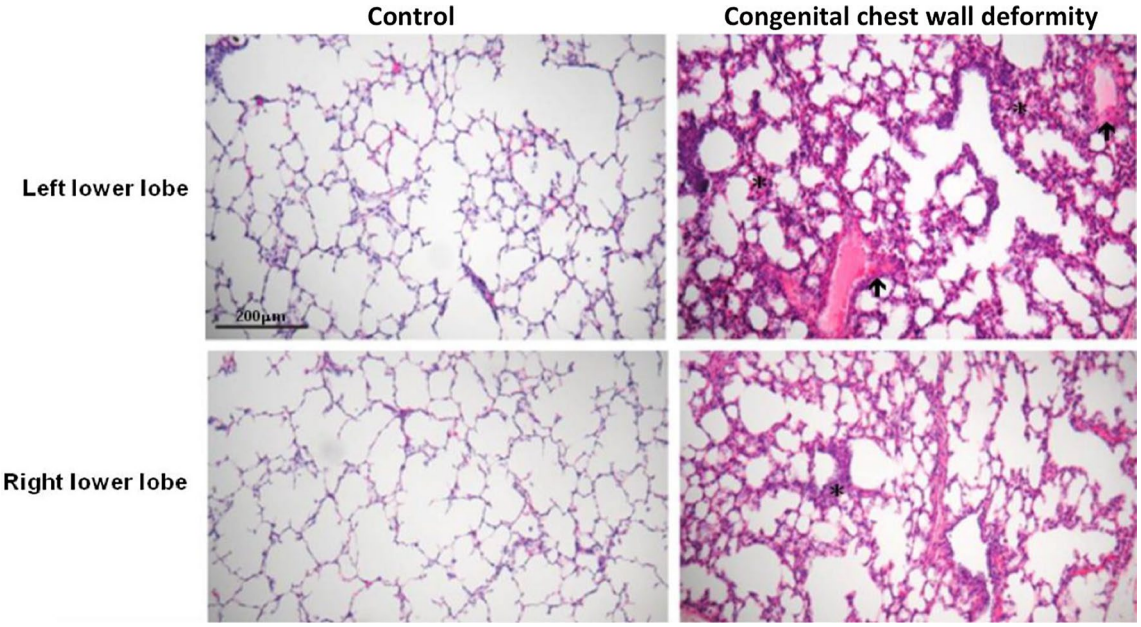

Fig. 2 Severe alteration of lung development following in utero creation of a congenital chest wall deformity (CWD) in an ovine model. Hematoxylin + eosin coloration of lung sections from one control lamb (left panel) and CWD lamb # 4 (right panel) is shown. The upper panels show lung sections from the left lower lobe, whereas the bottom panels show lung sections from the right lower lobe. Scale bar = 200 µm. All images were taken at the same magnification. Congenital chest wall deformity was responsible for increased intra-alveolar septal thickness (*) and decreased secondary septation, as well as increased muscularization of small pulmonary arteries (↑) in both lungs, with the left lower lobe being the most affected

with the unilateral tethering technique and a significant asymmetry between the right and left lung volume. In a further study, they evaluated the effect of expansion thoracoplasty in their model [7] and found that despite some correction of the scoliosis, there was only a minimal increase in volume of the affected lung accompanied with a small increase in alveolar dimensions. Their results hence did not bring any evidence of improved alveolarization and lung function with treatment. Of note, the rib-tethering procedure was performed in seven-week-old rabbits and the corrective procedure occurred 3 weeks later. More recently, the same group created a spine deformity in 3-week-old rabbits and assessed the effect of an expansion thoracoplasty performed at 7 weeks versus 11 weeks [8]. Although the natural progression of the deformity was halted in both groups, the authors observed benefits on pulmonary hypoplasia in rabbits with the most severe deformity only, at the expense of further impairment of chest wall elasticity. Of note, while alveoli continue to form until adulthood in the rabbit, the bulk of alveolarization occurs in the first two postnatal weeks [9]. Hence, alveolar formation is well advanced at 7 weeks postnatally, therefore diminishing the impact of any surgical correction of thoracic deformity on lung development and function.

Following resection of two left ribs between days 70 and 75 of gestation, we observed variable severities of spine and CWD at birth including cases with lung hypoplasia seen on histological analysis. This represents a spectrum of disease that tends to mimic what is seen in humans as not all deformities seen at birth are severe. While none of the operated lambs was seen to have grown ribs again in the first two postnatal days, this is likely due to the lack of sufficient follow-up postnatally. Our model is unique because the deformity is initiated in utero and seen at birth with a wide range of deformities. To our knowledge, this approach has not been previously described.

Moreover, our new ovine model was seen to produce a thoracic insufficiency syndrome at birth. This is evidenced by one lamb with a severe CWD that presented with respiratory distress syndrome. Hence, our CWD model provides a platform to study the impact of spine and chest wall deformities on lung function as a spectrum of disease.

However, this current study does not provide insight on the entire scope of congenital spine and chest wall deformities as well as its natural history. Congenital scoliosis in its entirety could not be studied using this model, as it does not replicate a spine and chest wall deformity that is caused by defects of segmentation. In addition, the deformity of the thoracic cage was not fully defined as only Cobb angles were measured. The deformity of the chest wall should be further evaluated with chest CT scan in future works. Moreover, the natural history of this

disease was not studied as the lambs with severe deformity were euthanized at birth after ventilator studies were performed.

In addition, it is still difficult to assess why only 4 out of the 6 lambs had only mild spine and chest wall deformity. There were no clear explanations as surgical technique were similar in all cases as well as prenatal care of the gestating ewes. Hence, the etiology of a spine and chest wall deformity cannot be simply explained by the excision of two ribs.

The above questions exceed the scope of the present study and are potential areas for future research. Our study aimed to provide a new model of spine and chest wall deformity present at birth that mimics as closely as possible what is seen in humans. The natural history, clinical impact as well as potential treatment modalities need to be addressed in future studies, and we believe that our model can provide the basis of these studies as we have produced a wide spectrum of spine and chest wall deformity present at birth. A study with more lambs also needs to be done in order to obtain not only enough statistical significance, but also surviving lambs that have significant deformity.

Measurements of respiratory system mechanics

The simple removal of two ribs resulted in a mean decrease in respiratory system compliance of 60% and in inspiratory capacity of 39% compared to controls. Severity of the decrease in lung function grossly paralleled severity of the spinal deformities (Table 2).

In addition, histomorphometrical analysis performed in one lamb with severe CWD as a proof of principle suggests that alteration in lung mechanics is associated with significant delay in lung alveolarization (Fig. 2).

However, two of the lambs with congenital wall and spine deformity (CWD 1 and 2) were not included in the final analysis for postmortem measurements. This was done as these lambs were euthanized 10 days later and would introduce additional bias to our results. The delay was unfortunately due to the fact that these lambs were born later than the other lambs.

Moreover, the pathological laboratory that performed the histomorphometrical analysis was not equipped, nor had the ability at that time to analyze and quantify the number of alveoli. Furthermore, since the context of this initial study was to prove a concept, the study was not designed to include a complete and detailed histological analysis. In addition, the position of the diaphragm and spine of a quadrupedal model is different than in bipedal humans and these effects on respiratory mechanics remain undefined. Theses are all limitations to our study that should be addressed in the future.

Conclusion

This a feasibility study of a new ovine model of spine and chest wall deformity observed at birth. The model presents a wide spectrum of spine and chest wall deformity, including one newborn lamb with thoracic insufficiency syndrome, as seen in humans. This model lays the groundwork for future studies aiming to assess the natural history and the clinical impact of a spine and chest wall deformity present at birth on lung function and development.

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Compliance with ethical standard

Conflict of interest SP received Royalties and consultancy outside the submitted work from EOS-Imaging; he is also stockowner of Spinologics; he received honorarium and consultancy from K2M, DePuy Synthes Spine and Medtronic. His institution received grants from: DePuy Synthes Spine, Canadian Institutes o Health Research, Pediatric Orthopedic Society of North America, Scoliosis Research Society, Medtronic, EOS imaging, Canadian Foundation for innovation, Setting Scoliosis Straight foundation, Natural Sciences and Engineering Council of Canada, Fonds de recherche Québec-Santé, Orthopaedic Research and Research Education Foundation. His institution also received fellowship support from Medtronic and DePuy Synthes. JS received an educational grant from Fonds de recherche du Québec- Santé and he is also receiving a salary from Régie de l'assurance maladie du Québec. NS, GG, SB and BP have nothing to disclose. JP received the research Chair in Neonatal Respiratory Physiology and a grant from the Canadian Institutes of Health Research.

Ethics The ethics committee for animal care and experimentation of the Université de Sherbrooke approved the study.

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