Published in final edited form as:

Spine Deform. 2019 March ; 7(2): 213–219. doi:10.1016/j.jspd.2018.07.002.

Creation of a Porcine Kyphotic Model

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Abstract

Study design: Large animal study.

Objective: Create a thoracic hyperkyphotic deformity in an immature porcine spine, so that future researchers may use this model to validate spinal instrumentation and other therapies used in the treatment of hyperkyphosis.

Summary of background data: Although several scoliotic animal models have been developed, there have been no reports of a thoracic hyperkyphotic animal model creation in an immature animal. The present study was designed to produce a porcine hyperkyphotic model by the time the pig weighed 25 kg, which corresponds to the approximate weight of a child undergoing surgery for early onset scoliosis (EOS).

Methods: Successful surgical procedures were performed in 6 consecutive 10 kg (male, 5 week old) immature Yorkshire pigs. Procedure protocol consisted of 1) a left thoracotomy at T10-T11, 2) screw placement at T9 and T11, 3) partial vertebrectomy at T10, 4) posterior interspinous ligament transection, and 5) placement of wire loop around screws and tightening. Weekly x-ray imaging was performed preoperatively and postoperatively, documenting progressively increasing kyphosis as the pig grew. Necropsy was performed 5-6 weeks after surgery, with CT, slab section, and histologic analysis.

Results: Average T9-T11 kyphosis (measured by sagittal Cobb angle) was $6.1 \pm 1.4^\circ$ (mean $\pm$ SD) preoperatively, $30.5 \pm 1.0^\circ$ immediately postop, and significantly increased to $50.3 \pm 7.2^\circ$ ($p < 0.0001$) over 5-6 weeks in 6 consecutive pigs at time of necropsy.

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Conflict of Interest:

None of the authors of this paper have a conflict of interest that might be construed as affecting the conduct or reporting of the work presented.

IACUC Approval:

This study was approved by the Institutional Animal Care and Use Committee at MUSC.

Conclusions: An animal model of relatively more rigid appearing thoracic hyperkyphotic deformities in immature pigs has been created. Subsequent studies addressing management of early onset kyphosis with spinal instrumentation are now possible.

Level of evidence: V

Keywords

early onset scoliosis; thoracic hyperkyphosis; animal model; immature spine; pig

INTRODUCTION

Over the past decade, management of early onset scoliosis has been refined, and satisfactory results are routinely reported. However, in patients with thoracic hyperkyphosis, complications are frequent and results are less satisfactory [1-3]. These ongoing problems warrant creation of modeling systems as venues for validating surgical techniques and instrumentation for children with thoracic hyperkyphosis. While *ex vivo* biomechanical testing with cadaveric spines and computer modeling for spinal instrumentation can be useful, the present study focuses on developing an *in vivo* animal model.

In 1962, Langenskiöld first published the creation of an animal model for scoliosis by resecting ribs in rabbits [4]. Subsequently, there have been almost a hundred publications on scoliotic animal models in pigs, goats, sheep, chickens, and mice [5]. The most common method involves creating a tether on lateral vertebral bodies, relying on subsequent asymmetric growth to produce a scoliotic deformity [6]. There are a few reports describing kyphotic deformity animal models in the cervical and lumbar spines. Pekmizci and coworkers created a lumbar kyphotic deformity in 8 week old pigs by an anterior fusion and tethering procedure, with an average kyphosis of 49° at maturity [7]. The emphasis of their work was the evaluation of kyphotic deformities on the size of the spinal canal. Kong *et al* used a laminectomy to create a cervical kyphotic deformity in sheep [8]. Zheng *et al* used an offset tether to create kyphoscoliotic deformities in pigs [9]. Oda *et al* created lumbar kyphotic deformities in sheep to examine the effect of fixed kyphosis on adjacent motion segments [10]. Shimizu *et al* created cervical kyphotic deformities in Japanese small game fowls to assess the effect of kyphosis on the spinal cord [11]. All of these model systems were useful, but none created a deformity in the thoracic spine of an immature animal, simulating thoracic hyperkyphosis in a child.

The objective of the present study was to create a porcine thoracic hyperkyphotic deformity so that subsequent studies could evaluate the effect of corrective instrumentation and other therapies on the immature spine. Yorkshire pigs were selected for this study due to their relatively similar size and shape compared to the human spine [12]. In addition, pigs show large spinal growth potential in their first 6 months of life, making them ideal for a spinal deformity animal model. The model outlined in this paper established a thoracic hyperkyphotic deformity at the time the weight of the pigs was 20-25 kg, comparable to the weight of a 7 year old child, the average age of initial surgery in recent studies on early onset scoliosis [13,14]. Furthermore, the kyphotic deformity was created at a developmental stage

at which the pigs still had considerable future spinal growth remaining, allowing for accurate future validation of the effect of surgical instrumentation on an immature spine.

MATERIALS AND METHODS

Husbandry

All procedures were approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina (MUSC). Domestic Yorkshire farm pigs (male, 5 week old, 10-11 kg; n=15) were acquired one week before surgery from a class A USDA-registered swine vendor (Valley Brook Research, Madison, GA). This size was selected to ensure that the pigs were sufficiently mature to survive a thoracotomy and small enough that the created deformity would be established by the time they weighed 25 kg, a weight that would provide a reasonable counterpart to the size of many children undergoing surgery for early onset scoliosis [13,14].

All pigs received a broad spectrum antibiotic (Excede; 5 mg/kg, IM) for prevention of post-shipping respiratory disease. Pigs received ad lib reverse osmosis water, twice daily measured commercial diet, rotated enrichment devices and an automated 12:12 light:dark cycle. A liquid diet (Ensure®, Abbott Laboratories, Abbott Park, IL) was fed the day before surgery to reduce the amount of ingesta in the gastrointestinal tract compressing the diaphragm cranially toward the surgical site. The pigs were bathed 1-3 days before surgery using chlorhexidine soap, ensuring contact time was at least 10 minutes before rinsing. The pigs were then fasted overnight with constant access to water.

Anesthesia

The pigs were pre-medicated subcutaneously with atropine (0.04 mg/kg), acepromazine (1.1 mg/kg) and ketamine (22 mg/kg). They were then induced with isoflurane in oxygen administered by face mask and intubated with a cuffed endotracheal tube (5.0 mm internal diameter) for maintenance. Combination preemptive analgesia was administered subcutaneously (sustained release buprenorphine; 0.24 mg/kg) and intramuscularly (carprofen; 2 mg/kg). A 1-inch, 22-gauge intravenous (IV) catheter was placed in a marginal ear vein and secured for administering sterile crystalloid fluids (10 mg/kg/hr) using a fluid line warmer and volumetric infusion pump throughout surgery. Whole blood was collected for measurement of baseline packed cell volume and total solids. Cefazolin (62.5 mg/kg; IV) was given prophylactically since orthopaedic screws and wires were chronically implanted.

Intraoperative monitoring included continuous electrocardiogram, heart rate, esophageal temperature, and pulse oximetry for oxygen saturation (SurgiVet Advisor, Smiths Medical PM Inc., Waukesha, WI). Pigs were mechanically ventilated using a calculated tidal volume of 10 ml/kg, and end tidal CO₂ was maintained between 30 and 40 mmHg by adjusting airway pressure (15-25 cm H₂O) and respiratory rate (12-20 breaths/min). Pigs were kept warm with a circulating warm water heating blanket and a convection warmer.

Lateral Thoracotomy

Before a successful model system was developed, two unsuccessful attempts were made. The procedures attempted to replicate successful techniques for creating scoliosis animal models by either performing an epiphyseodesis, or creating a tether. A left lateral thoracotomy was performed at the 9th-10th intercostal spaces, and the ribs were distracted with a small Finochietto retractor. Procedures were performed on T9-T11, where the least retraction on the immature porcine lung was necessary. The first procedure was an epiphyseodesis, with bone graft placed in the anterior disc spaces. This procedure was unsuccessful, as it created minimal kyphosis. For the next attempt, a tethering technique, similar to that described by Newton for creating a scoliotic animal model [15], was performed. Screws were used in the anterior surface of T9 and T11 vertebral bodies, with a loop of #20 stainless steel wire placed around the screws in tension to create a tether. This was also ineffective.

Subsequently, the successful procedure was developed using a combination of techniques. A left lateral thoracotomy was performed at the 10th-11th intercostal spaces (Figure 1) and the anterior vertebral bodies were exposed (Figure 2). Anteroposterior screws were placed at T9 and T11, as far removed as possible from T10 to preserve a strong anterior cortex for secure purchase (Figure 3A). Through a separate dorsal incision, an open release of the supraspinous and interspinous ligaments was performed from T9-T11. Then, a partial vertebrectomy of the anterosuperior and anteroinferior sections of T10 was performed (Figure 3A). A wire loop was then placed around the screws and tightened, compressing the anterior T9-T11 vertebral bodies (Figure 3B). Fluoroscopic images were acquired using a c-arm intraoperatively and postoperatively (Figure 4).

Postoperative care

Analgesia was provided using a fentanyl patch, 5 µg/kg applied to the distal forelimb and a single dose of buprenorphine, 5 µg/kg. Pigs received intra-operative IV fluid support at a rate of 10 ml/kg/hour and were ventilated at a rate of 10-12 ml/kg using end-tidal CO₂ measurements to make ventilator adjustments. After surgery, the pigs were maintained in the animal research facility at MUSC and allowed to grow until they were approximately 10-11 weeks old and 20-25kg.

Radiographic Evaluation

Sagittal fluoroscopic images of the thoracic spine were acquired intraoperatively, immediately postoperatively, and at one week intervals for 5-6 weeks until necropsy. With each radiograph, thoracic kyphosis was measured (in degrees) between segments T9-T11 using the standard Cobb angle technique (Figure 4). Pigs were euthanized 5-6 weeks post op. At necropsy, the spine was dissected and most skeletal muscle was removed. Computed tomography scans were obtained and three-dimensional sagittal reconstructions were generated (Figure 5A).

Histologic evaluation

Spinal segments from T8-T13 were immersion fixed in Cal-Ex II for fixation and decalcification. Screws were removed from the vertebral bodies post-fixation, and spine was

hemi-sectioned longitudinally. A 3.0 mm thick slab section was removed from the cut face and subsequently bisected to allow placement into tissue cassettes (Figure 5B). Tissues were paraffin embedded, with 5 μ m thick sections placed on negatively charged glass slides and stained with hematoxylin and eosin. Sections were examined and digitally reconstructed subgross images were captured.

Statistical Analysis

The measured kyphotic angles were reported as mean $\pm$ standard deviation. One-way ANOVA and Tukey's post hoc tests were performed in SPSS to determine the effect of post-operation time on kyphotic angle. Statistical differences were reported at p-values < 0.05 .

RESULTS

Perioperative complications

The initial epiphyseodesis (1 case) and anterior tethering procedure (1 case), when used in isolation, failed as they created only minimal kyphosis. Based on the study of Pekmici et al [7], where they describe the entire growth period of the pig was necessary to create a 49° kyphotic deformity, we concluded that there was no adequate time to create a $>30^\circ$ kyphosis by the time the pig's weight was 20-25, Kg, and therefore we rejected these methods. In addition, the following complications were encountered before the reliable procedure was perfected:

- 1) Intraoperative tachycardia and rapid respiratory rate, subsequently determined to be secondary to selenium deficiency (2 cases).
- 2) Subacute vertebral osteomyelitis at operative site, also in a pig determined to have selenium deficiency (1 case).
- 3) Screw penetration and paraplegia (1 case).
- 4) Canal penetration during partial vertebrectomy causing paraplegia (1 case).
- 5) "Plowing" of screws resulting in inadequate screw purchase on vertebral body (2 cases). Subsequently, 6 consecutive successful procedures using the combination of anterior tethering, partial vertebrectomy, and posterior spinal ligament release were performed without complications.

Radiographic Data

Sagittal fluoroscopic images were obtained preoperatively, immediately postoperatively, and at one week intervals for 5-6 weeks until necropsy. Sagittal Cobb angles between T9-T11 for each pig were measured. The average preoperative kyphosis (measured by sagittal Cobb angle) was $6.1 \pm 1.4^\circ$ between T9-T11. The average kyphosis created at the time of initial surgery was $30.5 \pm 1.0^\circ$ ($p < 0.0001$ compared to preop) between T9-T11, with a significant increase to $50.3 \pm 7.2^\circ$ ($p < 0.0001$ compared to initial surgery) at the time that the pigs were euthanized, 5-6 weeks post-operatively (Table 1). Kyphosis between T9-T11 increased

gradually throughout the study (Figure 6). Reconstructions of CT images at necropsy confirmed the creation of kyphosis in the animals (Figure 5A).

Histologic evaluation

Histologic evaluation of spinal segments T8-T13 revealed fusion and stunted growth of the anterior growth plates between T9-T10 and T10-T11. The posterior growth plates in these segments remained intact and continued to grow relatively uninhibited (Figure 5C). This resulted in anterior wedging of the vertebral bodies and a hyperkyphotic deformity between T9-T11. Growth plates and intervertebral discs in segments adjacent to T9 and T11 remained intact.

DISCUSSION

Despite the challenges and high complication rates associated with the surgical treatment of thoracic hyperkyphosis in children, there are no reports of previous attempts to create a thoracic hyperkyphotic animal model in immature spines. The goal of the present study was to create such a model, so that future studies may use the model to evaluate and validate corrective instrumentation for the more rigid thoracic hyperkyphotic spinal deformities.

In our first attempt, an epiphyseodesis was performed at T9-T10 and T10-T11. The second attempt created a spinal tether placed anteriorly between T9-T11. Both of these techniques, when used in isolation, failed to produce the desired hyperkyphosis in the time allotted. This failure can likely be attributed to the biomechanics of quadruped weight bearing, which places a tensile force on the anterior porcine thoracolumbar spine. The initial phases of model development were also hampered by unanticipated intra-operative and post-operative complications subsequently attributed to documented Selenium/Vitamin E deficiency in piglets from a vendor that had previously supplied healthy animals. Two of these pigs developed intraoperative tachycardia and tachypnea, and one pig developed vertebral osteomyelitis. Selenium and Vitamin E deficiency can have multiple negative consequences including hemolytic anemia, neurological deficits, muscle weakness, and immune compromise [16,17]. The severe deficiency resulted in the cardiopulmonary complications and vertebral osteomyelitis observed in some of our pigs. When a new vendor was selected that had piglets with Vitamin E/selenium levels within established lab reference ranges, these problems went away.

In addition, during the pilot procedures two cases of paraplegia also occurred. The procedure that was ultimately effective is technically demanding. The AP diameter of the midportion of the T10 vertebral body in a 10 kg pig is only about 12 mm. This requires precise placement of the screws, and careful attention to instrument placement during the vertebrectomy. The reported complications occurred during the learning curve for the procedure performed on the 6 consecutive pigs. The IACUC was advised on a timely basis of all complications.

The successful technique that was established utilized an approach that combined T9-T11 tethering, partial vertebrectomy, and open release of the supraspinous and interspinous ligaments between T9-T11. With this technique, the kyphosis (measured by sagittal Cobb angle) between T9-T11 increased by an average of 20° between creation and necropsy 5-6

weeks later. This increase of kyphosis can be attributed to the undisturbed posterior portion of the T9-10 and T10-11 growth plates. The partial vertebrectomy on the anterosuperior and anteroinferior portion of T10 creates a 30° kyphosis between T9-T11 at the time of initial surgery, when the anterior spine is tethered by the wire loop placed around the screws at T9 and T11. The posterior ligament release increases the flexibility of the spine by removing the posterior tether at this level.

Technical details of importance include accurate location of the thoracotomy at ribs T10-11 where minimal retraction of the lung is necessary. The immature porcine lung is friable and easily forms blebs, and successful repair of any disruption of the delicate pleura is difficult. Optimal screw placement is in cortical bone just adjacent to the T8-9 and T11-12 discs, where 14 mm screws can be used. The AP diameter of the midportion of the vertebral body is approximately 12 mm. It is advisable to tilt the screw tips slightly towards T10. If the screw tips are tilted away from T10, plowing of the screw is likely, with loss of cortical purchase.

There are limitations to this animal model that should be acknowledged. The weight bearing forces on the pig place a tensile force on the anterior spine, which is not the case for the human spine. This limitation could potentially be avoided by using bipedal primates, but the technical, ethical, and financial considerations involved in such a model are prohibitive. Of all quadrupeds used for spine research, the porcine spine most closely anatomically resembles the human. Our model most closely resembles clinical congenital kyphosis (Figure 7), although some syndromes, such as neurofibromatosis, are associated with short, rigid deformity. Since these rigid deformities are more difficult to correct, we consider this resemblance advantageous for further studies of the response of the immature kyphotic spine to corrective instrumentation.

Spontaneous kyphosis has been noted in some Danish pig herds at T14-15, with dysplasia of the anterior growth plates responsible for development of the deformity [18]. However, this model for spontaneous kyphosis is unreliable and has a maximum incidence rate of 2-3%. Furthermore, it primarily generates thoracolumbar kyphosis and rarely goes more proximal than T14-15. Our model allows for the creation of kyphosis closer to the mid thoracic region. Recent work in our laboratory has documented the hypermobility of the porcine lumbar spine with junctional kyphosis resulting from every attempt to instrument the thoracolumbar spine. Therefore, the more proximal location of our kyphotic model could make the study of corrective instrumentation possible by allowing the thoracic spine alone to be instrumented.

In conclusion, this is the first report describing the successful creation of a large animal thoracic hyperkyphosis model in an immature animal. The weight of the pigs at the time of necropsy was 20-25 kg, which corresponds to the average weight of children undergoing surgery for early onset scoliosis. The hyperkyphotic curve was established at a time when the Yorkshire pigs still have considerable growth remaining. Thus, this animal model could allow researchers to validate instrumentation utilizing an immature kyphotic spine, closely resembling the size of the spine of children undergoing treatment of early onset kyphotic deformity.

Acknowledgements:

This project was supported by a research grant from the Scoliosis Research Society.

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KEY POINTS

- While many spine deformity animal models have been reported in the literature, there have been no successful models that have created a thoracic hyperkyphotic deformity in an immature spine.
- An animal model was developed that creates a thoracic hyperkyphotic deformity in an immature porcine spine by utilizing a tether at T9-T11, partial vertebrectomy, and posterior interspinous ligament release.
- This animal model can be used in future studies to investigate corrective instrumentation for hyperkyphosis in immature spines.

Figure 1.
Localizing site of thoracotomy prior to incision (**1A**). Site of thoracotomy (**1B**).

Figure 2.
Exposure of spine (**2A**). Control of segmental vessels and mobilization of aorta (**2B**).

Figure 3. Placement of screws and partial resection of T10 vertebral body (**3A**). Wire loop placed around screws and tightened (**3B**).

Figure 4. Imaging at completion of procedure. Lines indicate the measurement of the Cobb angle between T9-T11. In this example, the Cobb angle was 31° immediately postop.

Figure 5. 3D reconstruction of computer tomography images (**5A**) and slab section of deformity at necropsy (**5B**). Histology of kyphotic deformity (**5C**). The anterior portion of the T9-T11 growth plates was disturbed by the vertebrectomy and are partially fused (white arrow). The posterior portion of the growth plates remain active after initial surgery (black arrows). Growth plates and intervertebral discs in adjacent segments remain healthy.

Figure 6.

The average sagittal Cobb angle between T9-T11 was $6.1 \pm 1.4^\circ$ preoperatively. An average kyphosis (measured by sagittal Cobb angle) of $30.5 \pm 1.0^\circ$ was generated at the time of surgery and increased to $50.3 \pm 7.2^\circ$ at necropsy. A significant increase in kyphosis was observed throughout the course of the study. *: $p < 0.05$ compared to preop. . #: $p < 0.05$ compared to surgery.

Figure 7.
Comparison of necropsy specimen of porcine thoracic kyphotic deformity (left) with sagittal CT image of congenital thoracic kyphosis in a 9 year old child (right).

Table 1.

Weekly kyphosis measurements (in degrees) following procedures in 6 consecutive pigs. Radiographs not made at necropsy for pigs 1, 2, and 3. Necropsy values measured on slab section.

Pig ID	Preop	Surgery	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Necropsy
1	4.8	30	32	37	40	50	necropsy	--	62
2	6.2	29	35	36	37	37	40	necropsy	48
3	8.1	32	30	38	39	40	43	necropsy	53
4	5.9	31	33	37	35	35	41	43	48
5	4.3	31	33	30	35	33	36	43	40
6	7.0	30	30	33	35	37	50	49	51
Means	6.1	30.5	32.2	35.2	36.8	38.7	42.0	45.0	50.3
SD	1.4	1.0	1.9	3.1	2.2	6.0	5.1	3.5	7.2