Application of an Electric Current in the Developing Chicken Embryo Induces Spinal Deformity

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Introduction: Congenital scoliosis is a relatively rare condition, affecting 1 in 1000 live births, where vertebral anomalies present at birth involve either a failure of vertebral formation, such as a hemivertebra, or a failure of vertebral segmentation, such as a unilateral unsegmented bar, where different parts of the vertebral body and posterior elements can be affected to varying extent [4] creating complex spinal deformity. Congenital vertebral malformations result in unbalanced spinal growth, leading to deformities that can range from mild, asymptomatic curves to severe curves requiring surgical intervention in infancy [2]. Imaging of the spine pathoanatomy on bi-planar X-rays is described as scoliosis when projected onto the coronal plane and kyphosis when projected in the sagittal plane [1]. Congenital anomalies of the thoracic spine may involve rib cage deformities that result in thoracic insufficiency syndrome (TIS), where anatomic constriction of the thorax prevents uniform lung inflation and impedes lung growth, leading to extrinsic restrictive lung disease and increased childhood mortality [3]. The risk of spine deformity progression depends on various factors, including the morphology and anatomical location of the vertebral anomalies as well as the rate of spine growth. Given that little is known about the pathophysiology of congenital spine deformity, clinicians are unable to make informed treatment decisions. Our understanding of congenital spine deformity is limited by a lack of relevant animal models to study mechanisms contributing to congenital spine pathoanatomy. The purpose of this study is to examine the effects of applying targeted electric current on the formation and location of congenital spinal deformities in the developing chicken embryo.

Methods: 47 specific pathogen-free (SPF) eggs were incubated until embryonic day 3 (E3) when the shell was windowed for intervention. Electroporation parameters were set to 50V, with 50 ms pulses x 3 separated by a 950 ms interval. In the intervention group (n=42), an electrical current was delivered through electrodes placed above (n=11), below (n=12), and at an oblique angle to the vitelline vessels (n=19). Five eggs served as controls, with electrodes placed but no current applied. Eggs were sealed and incubated until E10, then the embryos were sacrificed. At E17, 11 eggs were assessed to specifically and accurately characterize the 3D nature of induced deformity using µCT, with 7 receiving intervention and seven controls. For histologic analysis, select specimens harvested at E10/E11 were sectioned and stained using both standard H&E and Alcian blue / Picrosirius red to observe morphological defects of the vertebrae at the apex of the deformity. To facilitate standardization, specimens were sectioned coronally through the spinal canal. Alcian blue was used to stain the vertebral cartilaginous anlagen, delineating the primitive spine from surrounding tissue. In the cohort of eggs assessed at E10/E11, descriptive statistics determined proportions for survival and deformity induction, at a 95% confidence interval, calculated using the Wilson score method. To account for the small sample size, overall survival proportions of treated vs control specimens were compared using Fisher's exact test. Additionally, among the surviving specimens, Fisher's exact test was used to compare deformity proportions between the treated and control groups ($p<0.05$).

Results: The electroporation protocol resulted in high survival rates (n=33/46, 72%) and high spinal deformity rates (n=29/33, 88%). Spinal defects (n=30/33, 90%) were region-specific, based on electrode placement (**Fig. 1**). All control embryos survived (5/5) without spine deformity (0/5), highlighting the significant difference in deformity rates between groups ($p<0.0001$, **Fig. 1**). Control chicks did not exhibit aberrant morphology of vertebral anlagen or curvature of the spine. µCT assessment of the inferior orientation group @ E17 identified unsegmented bars and block vertebrae at the thoracolumbar region. In scoliotic chicks, laterality comparisons were made between sides exhibiting aberrant vertebral development compared to the opposite side. Bilateral defects were observed in 4/9 specimens with fusion, and unilateral defects were observed in 5/9 specimens with fusion. Histologic evaluation of specific regions in representative specimens confirmed segmentation defects between adjacent cartilaginous vertebral anlagen, not present in controls **(Figs. 2&3)**.

Discussion: Since scoliosis can occur without vertebral malformations from changes in the disc or surrounding muscles, we verified that the deformities observed during dissection were due to bony abnormalities mimicking the vertebral defects seen in patients with congenital scoliosis. HE and Alcian blue staining of E10/E11 specimens revealed morphological abnormalities in the vertebral cartilaginous anlagen at the deformed region of interest, which were absent in controls. In the sectioned samples, fusions of adjacent vertebral segments were observed, indicating failure of segmentation as the cause of the pathoanatomy. These patterns were consistent across the targeted region and were not seen in areas of the spine not operated on, indicating local regional control of electroporation. A disruptive vascular pathogenesis has been widely hypothesized as a cause for the development of congenital spine anomalies, such as Klippel-Feil syndrome (5,6). During re-segmentation (membranous phase), pairs of dorsal intersegmental arteries arise from the dorsal aorta and supply blood to the respective somites. In a histological study by Tanaka et al, a relationship between abnormal intersegmental artery distribution and vertebral segmentation defects was observed in 40 of 266 human embryos and fetuses (5), where cells closest to the arteries differentiated more rapidly. During the membranous phase, obstruction or delay in the development of the vertebral arteries could cause ischemia and halt morphogenesis, leading to segmentation failure. The extent and location of vertebral fusion can be explained by the location and severity of the vascular lesion (6), which could be controlled through targeted electrode placement. We suggest that vascular injury is the mechanism inducing vertebral segmentation defects in this chick model, supported by mild bleeding events observed in our study when applying electric current. Although not all deformed specimens experienced a bleeding event, microvascular injury to the intersegmental arteries remains plausible and deserves further investigation.

Significance/Clinical Relevance: The ability to produce region-specific thoracolumbar malformations that persist through late-stage development provides a valuable platform for investigating disease progression, biomechanical consequences, and potential therapeutic interventions in a clinically relevant form of congenital scoliosis.

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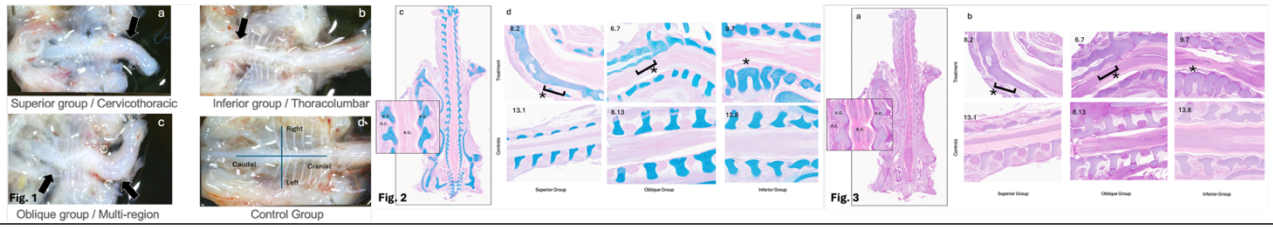

Fig. 1 Shows representative gross specimens from the dissection of the superior group (a), the inferior group (b), and the oblique group (c). Each display coronal plane structural curves. Control specimens (d) showed no signs of scoliosis. **Fig. 2 & 3** Display coronal H&E (a, b) and Alcian blue / Picrosirius Red (c, d) sections from the respective groups, illustrating abnormal vertebral morphology. In the superior group, sections are taken from the cervical/cervicothoracic region; in the oblique group, from the thoracic region; and in the inferior group, from the thoracolumbar region. Adjacent segments in the scoliotic region of interest show fusion of the vertebral cartilaginous anlagen, not seen in controls, indicating vertebral failure of segmentation as the structural cause of scoliosis observed at gross dissection. Sample normal, unoperated embryos (a, c) were used for relevant comparisons (s.c. (spinal cord); v.c. (vertebral cartilage); n.r. (nerve root); *Areas of abnormal morphology).