Chronic lung disease (also known as bronchopulmonary dysplasia or BPD) is a common malady in preterm infants that is associated with mechanical ventilation and supplemental oxygen. To discern the extent to which ventilation disrupts normal lung development, our team measured the amount of elastin (a protein that provides structure to airways and pulmonary arteries of lambs' lungs) using automated video thresholding of stain color. Also, through immunohistochemistry techniques, we calculated the relative concentration of fetal liver kinase-1 (Flk-1), a protein involved in angiogenesis (creation of blood vessels/vascular proliferation). BPD is when the lungs, and the elasins particularly, develop abnormally. One of the indicators of the disease is increased elastin deposition, which causes the tissue to appear more saccular and less lacy and branch-like than normal tissue. When an animal is ventilated, the expansion of the lungs exerts a stress that triggers greater elastin production (elastin being a major structural and hence stress-resistant component). To examine the extent to which this process occurs, we analyzed lung tissue from preterm, ventilated lambs and term lambs. By using automated video thresholding of stain color, we quantified the difference between the two groups regarding the amount of elastin in the tissue. As expected, we found that ventilated preterm lambs had a significantly greater concentration of elastin than the term lambs.

Another indicator of BPD is abnormal angiogenesis. To evaluate how ventilation affects angiogenesis, we determined the concentration of Flk-1, a receptor for vascular endothelial growth factor (VEGF) (the protein directly related to angiogenesis), in the tissue using immunohistochemistry. In immunohistochemistry, antibodies are used to bind to the protein of interest and then are marked with a stain. Thus, stained areas are where the protein is located, and the intensity of the stain indicates the protein's relative concentration. In our case, we first used an antibody made in a mouse to bind to Flk-1. Then we used an antibody made in a horse to bind to the mouse antibody. Avidin biotin complex was added to bind to the antibodies and to give a primary color. Diaminobenzidine (DAB) was used to provide a more visible, brown color to the antibody-complex. Finally, we stained the tissue with hematoxylin, a purple stain that colors the nuclei so we could see the relative positions the protein was located on or in the cells. Our results showed that the longer the lambs were ventilated, the lower the concentration of Flk-1. We interpreted this to mean that ventilation causes less Flk-1 to be produced, and hence, less angiogenesis to occur. One proposed mechanism is that hyperoxia, associated with the use of supplemental oxygen, causes down-regulation of the Flk-1 gene.

It is apparent, then, that mechanical ventilation with supplemental oxygen causes many problems that can lead to BPD. The force exerted on the lung through mechanical ventilation prompts excess elastin to be produced and to accumulate in the lung tissue, causing it to be more thick and saccular and so less efficient for gas exchange. In addition, the high concentration of oxygen that is required to maintain normal oxygenation causes down-regulation of the Flk-1 gene, thereby leading to decreased angiogenesis. This decrease in Flk-1 expression is accompanied by a parallel decrease in VEGF expression (as shown by previous studies). Therefore, blood vessel formation is not facilitated. In conclusion, it is important to discover all the ways in which mechanical ventilation affects lung development so as to find a means to remedy the problems it causes (like BPD) and deliver better medical care to pre-term infants.