

for initial cardiac evaluation. Once NRP was weaned, biochemical, hemodynamic, and echocardiographic assessments of cardiac function and metabolism were performed before organ procurement. DCD hearts initially reperfused with NRP demonstrated similar functional recovery following transplantation when compared to conventional beating heart donation preserved with cold storage. Following DCD, there is a severe decline in cardiac index, however, these organs demonstrated similar functional recovery post-transplantation when compared to conventionally transplanted hearts (beating heart donation followed by cold storage). Cerebral oximetry measurements dropped significantly after the withdrawal of life-sustaining treatments and remained stable throughout the NRP procedure. This confirmed the absence of adequate cerebral perfusion during NRP when the supra-aortic vessels were clamped. Lung compliance measurements throughout the experiments showed no significant changes from baseline during the NRP procedure.

**CONCLUSION:** This large animal model of DCD followed by thoracoabdominal NRP can be a reliable method to assess the cardiac function of a donor heart within the donor and establish if the organ can be transplanted. This protocol closely simulates the clinical scenario previously described for DCD and NRP in heart transplantation and has the potential to facilitate studies aimed at decreasing ischemia-reperfusion injury and enhancing cardiac functional preservation and recovery. It has the potential of being an adequate pre-clinical model that can be used to investigate novel pharmacologic and non-pharmacologic interventions that might improve cardiac functional recovery and have been previously validated only in small-animal models.

## P016 MITRAL VALVE CHORDAE TENDINEAE DEVELOP INDEPENDENTLY FROM LEAFLET TISSUE DURING FETAL DEVELOPMENT

M Martin, S Wells, J Chen

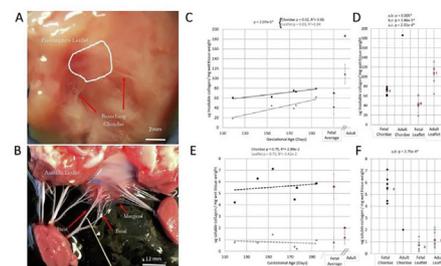
Halifax, Nova Scotia

**BACKGROUND:** During fetal development of the mitral valve, chordae tendineae are thought to derive from the leaflet after its delamination from the ventricular wall. However, preliminary work on the fetal bovine heart has shown the presence of branched chordae tendineae well before leaflet delamination (Fig. 1A). We hypothesized that the fetal chordae and leaflet tissue are developing along distinct timelines in the bovine heart. To test this hypothesis, we examined the anatomical development and extracellular matrix composition of the bovine mitral valve chordae and leaflet during fetal development and in adult.

**METHODS AND RESULTS:** Fetal bovine hearts were harvested from a local slaughterhouse and crown-rump length was used to determine gestational age. Samples ranged from 83-270

d.g. (full term = 290d.g.). Anterior leaflets were imaged then chordae number and leaflet area measured in ImageJ. Immature and mature collagen contents were determined using Sircol™ Soluble and Insoluble Collagen assays (Biocolour, Carrickfergus, UK). Age-paired tissue samples underwent Blyscan Sulfated Glycosaminoglycan (sGAG) assays (Biocolour) to determine sGAG content. Both chordae tendineae number and leaflet area increased linearly during gestation. While leaflet area continued to increase into adulthood the chordae number remained unchanged after birth. Mature, insoluble collagen content increased during gestation in both chordae and leaflet, with the rate of increase higher in the leaflet than chordae (ANCOVA  $p = 2.07e-5$ , Fig 1C). Despite this, average levels of mature collagen remained higher in chordae during gestation, with contents in the leaflet more than doubling post-natally (Fig 1D). Surprisingly, there was no change in the content of immature, uncrosslinked collagen during gestation in either tissue (Fig. 1E). Immature collagen was found at nearly 10-fold higher concentrations in fetal chordae than in leaflet, where low levels remained unchanged into adulthood (Fig. 1F). Unlike collagen, GAG content was similar in both tissues and remained unchanged during gestation and postnatal development.

**CONCLUSION:** Bovine mitral valve chordae tendineae and leaflet possess unique and independent developmental processes. Chordae increase in number along with leaflet expansion but only until birth, after which their numbers become fixed. In addition, collagen contents are strikingly different in both tissues and appear to be regulated by different mechanisms. The paradoxically low levels of immature collagen in the leaflet during fetal development suggests that the accumulation of mature collagen occurs via an upregulation of crosslinking. This parallels our observations in the maternal mitral valve, suggesting that valvular remodeling during pregnancy may be a recapitulation of fetal developmental mechanisms



**Figure 1** The bovine mitral valve chordae tendineae are forming independently of the leaflet during fetal development. **A**: Anatomical image of fetal and late fetal development show that branched chordae tendineae are present before the leaflet has formed. **B**: Fetal bovine mitral valve at 270 gestational days (full-term) showing that the chordae tendineae have differentiated into their different types (leaflet chordae). **C**: Representative images of insoluble (mature) and soluble (immature) collagen in the leaflet and chordae tendineae over gestational days. **D**: Scatterplot depicting variation in collagen concentrations and ANCOVA comparisons of overall average collagen concentrations between fetal/adult tissues. Black circles denote raw fetal chordae data ( $n=5$  months), white circles, grey triangles denote raw fetal leaflet data ( $n=3$  months), white squares, white circles, and red circles denote fetal and adult average (chordae  $n=3$ , leaflet  $n=19$ ). P-values with distinct letters are significantly different. **E**: Insoluble collagen concentrations increase in both the fetal chordae ( $n=16$ ,  $p<0.05$ ) and leaflet ( $n=18$ ,  $p<0.05$ ) during gestation. ANCOVA analysis reveals there is a significant difference between the fetal chordae and leaflet regression slopes ( $p<0.05$ ). **F**: The average soluble collagen concentration was greatest in the fetal chordae data in the leaflet ( $p<0.05$ ). Compared to the adult leaflet, the insoluble collagen concentration was significantly lower in the fetal chordae and leaflet ( $p<0.05$ ). Soluble collagen concentrations did not change during gestation in the fetal chordae and leaflet ( $p>0.05$ ). The average soluble collagen concentration was greatest in the fetal chordae than in the leaflet ( $p<0.05$ ). Compared to the adult leaflet, the soluble collagen concentration was not significantly different in the fetal leaflet ( $p>0.05$ ).

Natural Sciences and Engineering Research Council (NSERC),  
Vanier Canada Graduate Scholarship