Undergraduate Students - Population, Health & Wellness

Unraveling Gene Expression Profiles of Cardiac Genes That Participate in Embryonic Development of Congenital Heart Defects Using Chick Embryo

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ABSTRACT

Hypoplastic left heart syndrome (HLHS) is a rare but serious subtype of congenital heart defects (CHDs) at which the hemodynamics are disturbed. In this project, HLHS was introduced surgically by left atrial ligation (LAL) to embryonic chicks and the subsequent effects of it were studied. Different tests were done post-LAL to study cardiac morphology, function, and gene expression of cardiac markers.

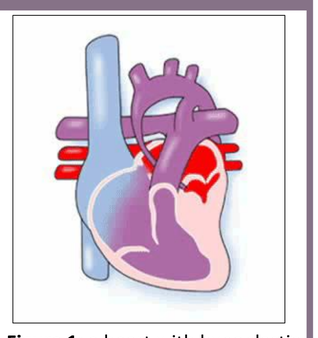

Figure 1: a heart with hypoplastic left heart syndrome (HLHS)

INTRODUCTION

- Congenital heart defects (CHDs) are heart disorders that may arise when cardiogenesis is disturbed. CHDs affects 1 – 1.2 % of newborns and considered as the primary cause of death in children under the age of one year.
- Hypoplastic left heart syndrome (HLHS) is a severe subtype of CHDs where the left ventricle volume is remarkably reduced. Thus, the heart will not be able to support the systemic circulation.
- The etiology of most CHDs cases believed to be because of disturbed hemodynamics.
- LAL is a surgical intervention that induce HLHS in vivo.
- Chicken embryos are commonly used animal models as it resembles the configuration of the four chambers and the four valves of human.

METHODOLOGY

Chick embryo culture

Fertilized eggs were incubated under 37.5℃, 60% humidity, and continuous rocking, the eggs were opened at ED3.

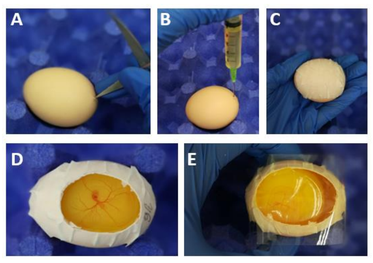

Figure 2: Egg opening on ED3 steps to access the embryos.
A: a small hole is made on the blunt side of the egg. **B:** 5 ml of albumin is removed. **C:** The top of the egg is covered with a tape. **D:** a small window is made on the top of the egg with sterile scissors. **E:** the window is sealed with transparent tape.

Introducing HLHS via Left Atrial Ligation (LAL)

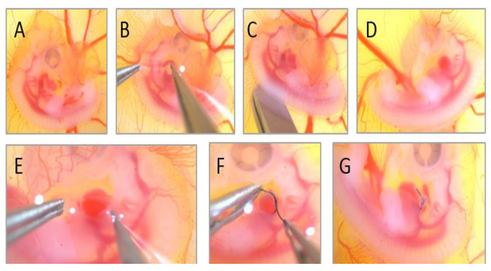

Figure 3: LAL steps on ED 4: A. chick embryo in HH23. **B:** Opening embryonic chorionic and allantoic membranes. **C:** flipping the animal **D:** the left side of the embryo. **E:** opening the pericardium **F:** placing the 10-0 knot on the top of the atrium. **G:** the animal after tightening the knot.

Heart function assessment via echocardiography

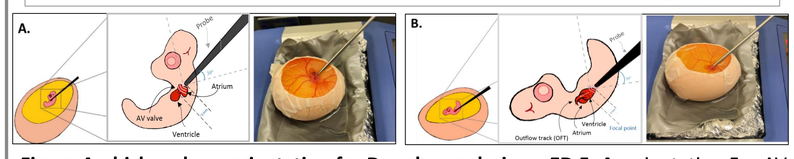

Figure 4: chick embryo orientation for Doppler analysis on ED 5. **A.** orientation For AV valve blood flow velocity measurement. **B.** orientation For OFT blood flow velocity measurement

Full heart isolation

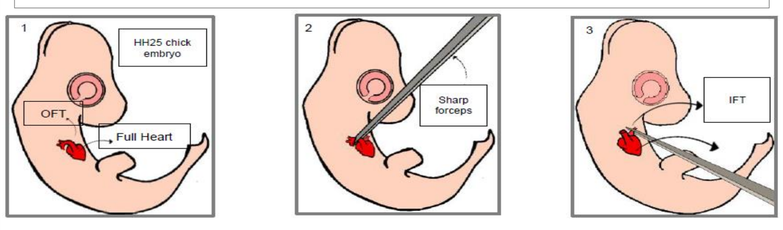

Figure 5: Full heart extraction steps on ED 5, 24-hr post-LAL: 1. The embryo is taken, OFT and IFT are located. **2.** Using sharp forceps the OFT is cut. **3.** The heart is flipped to expose IFT and cut. The heart is detached and kept in -80 for RNA isolation or in 4%PFA to be fixed for histology.

Relative gene expression of cardiac markers

RNA isolation and estimation, cDNA synthesis, RT-PCR

Histology

- Paraffin-embedded tissue sectioning
- Hematoxylin-Eosin (H&E) staining

RESULTS

Effect of LAL on heart morphology

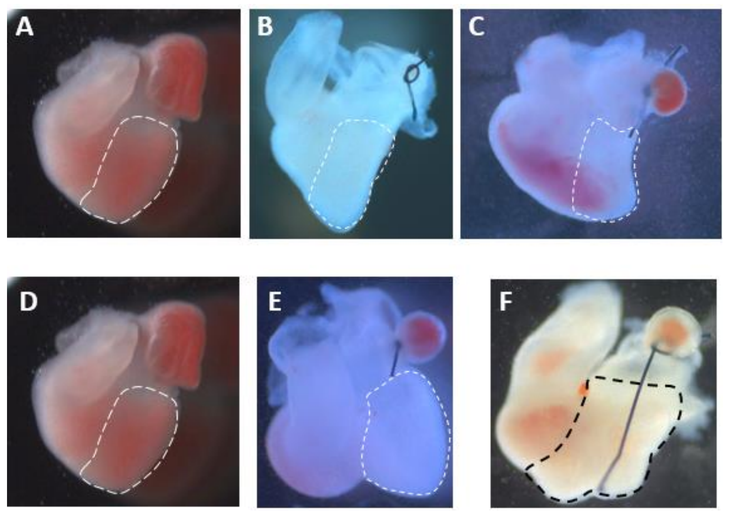

Figure 6: Heart LV size reduced and may develop to different shapes between ED5 chick embryos. A: control heart showing normal heart size with a normal LV size. **B** and **C**: hearts 24-hr post-LAL (HH25) showing decreased LV size. **D:** control heart showing normal heart shape. **E:** a malformed heart where the LV is small and abnormally pointy. **F:** LAL heart where the LV is malformed with 2 tips as compared to 1 tip.

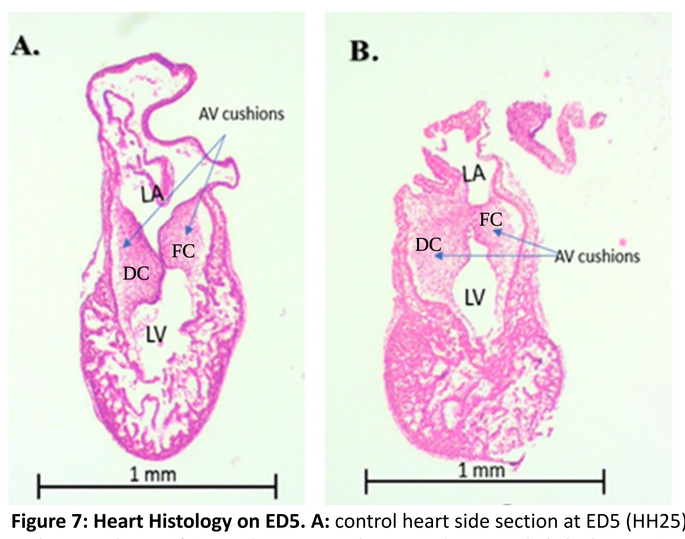

Figure 7: Heart Histology on ED5. A: control heart side section at ED5 (HH25) with normal size of AV cushions, LV volume, and myocardial thickness. **B:** a side section of embryonic chick heart 24-hours post-LAL (ED5) showing different AV cushion size, smaller ventricle, and thicker myocardium as compared to the control. DC= Dorsal cushion, FC= Front cushion.

Effect of LAL on heart function

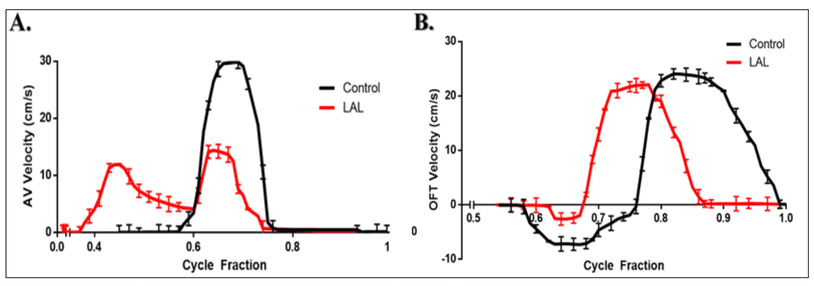

Figure 8: HH27 ED5 AV canal (A) and OFT (B) velocity profiles. Peak AV velocity significantly decreased for LAL heart. No significant change in OFT peak velocity. Duration time in AV valve significantly increased. In the OFT signal, the whole profile changed after LAL, the wave started earlier than control, and the backflow shown in the beginning of the control OFT signal disappeared in the LAL.

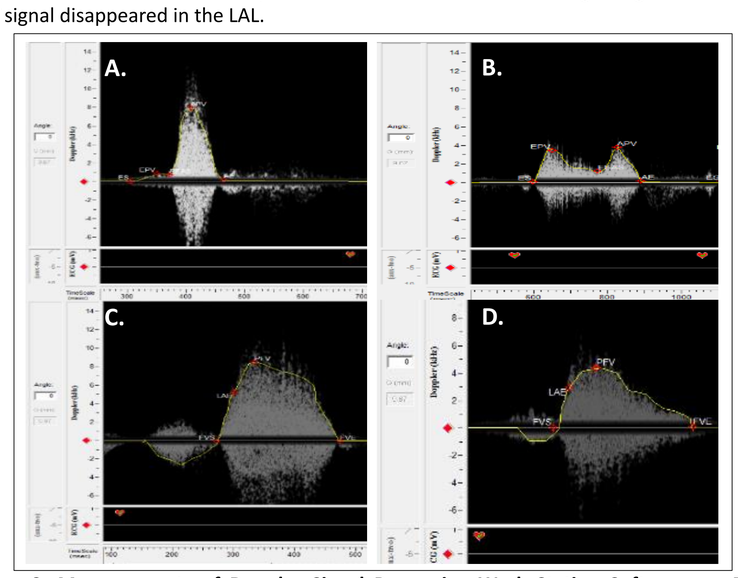

Figure 9: Measurements of Doppler Signal Processing Work Station Software on ED5. Hemodynamics were analyzed to detect AV signal in **A.** control and **B.** LAL and detect the OFT signal in **C.** control and **D.** LAL.

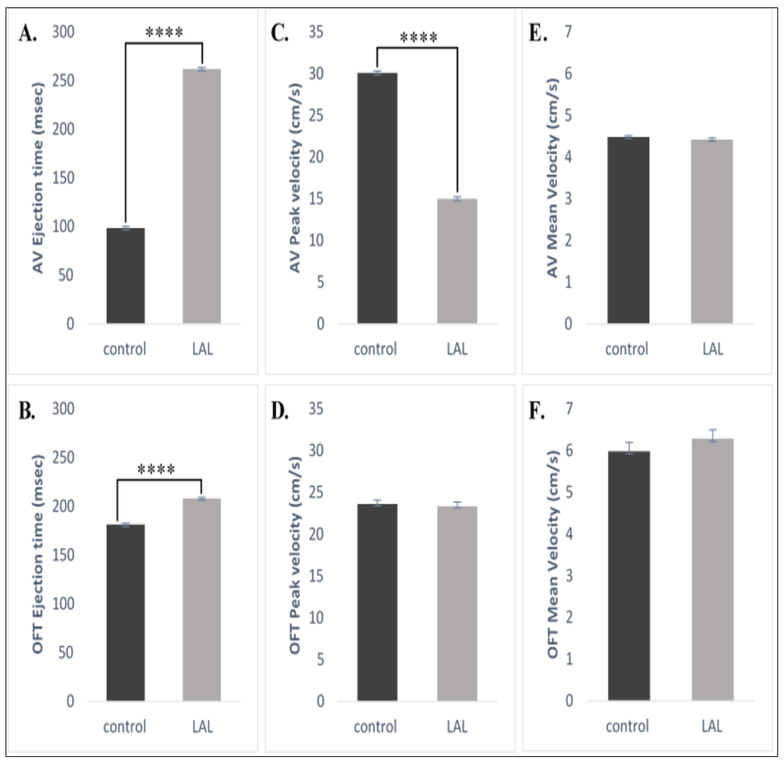

Figure 10: AV and OFT hemodynamic parameters on ED5. Doppler analyzed ejection time **(A, B),** peak velocity **(C, D)** and mean velocity **(E, F).** The ejection time in the AV **(A)** and the OFT **(B)** was significantly higher for LAL group. AV peak velocity was significantly lower in the AV **(C)**, but no significant change in OFT **(D)** of the LAL group. Analysis done by student t-test and data presented as mean ± SEM. N= 8 for control group and 12 for LAL group. The significant marks reflect the standard error.

Effect of LAL on cardiac markers gene expiration

LAL group has alterations in the expression level of all cardiac markers as compared to normal chick embryo hearts. LAL has significantly decreased the expression level of VEGF-a, TGF-b, BMP2, NKX2.5, and KLF2. No significant effect was shown of ACTA2.

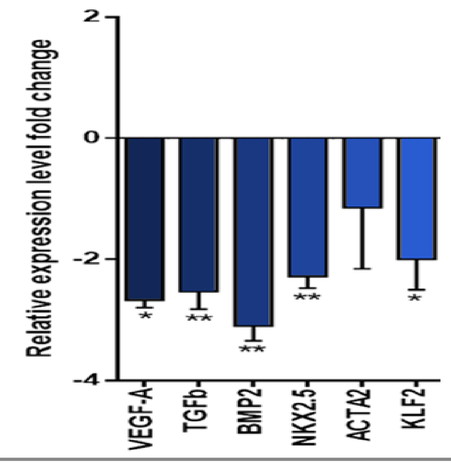

Figure 11: Relative gene expression level fold changes of different genes from LAL HH25 full hearts RNA extracts using RT-PCR. LAL led to significantly lowered level of most cardiac markers and shear stress markers. The expression was normalized to 18S gene expression. Analysis was done by two-way ANOVA, and the data is presented as mean fold change.
* = significant effect ($p<0.05$), ** = highly significant effect ($p<0.01$).

CONCLUSION

- Disturbing hemodynamics can lead to drastic malformations in the embryonic heart morphology.
- Disturbed hemodynamics negatively affect cardiac parameters and alter the heart ability to function.
- Changing hemodynamics leads to significant decrease in cardiac development markers expression level
- These findings put the starting to understand HLHS as a disease and think of candidate therapeutic techniques.

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