University of Cincinnati

Date: 1/17/2017

I, Tarek Alsaid, hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
THE EFFECT OF FETAL HEMODYNAMICS ON FETAL GROWTH IN SINGLE VENTRICLE AND TRANSPOSITION OF THE GREAT ARTERIES Fetuses

Student’s name: Tarek Alsaid

This work and its defense approved by:

Committee chair: Erin Nicole Haynes, Dr.P.H.

Committee member: James Cnota, M.D.

Committee member: Eileen King, Ph.D.
The Effect Of Fetal Hemodynamics On Fetal Growth In Single Ventricle And Transposition Of The Great Arteries Fetuses

A thesis submitted to the
Graduate School of the University of Cincinnati in partial fulfillment of the
requirements for the degree of Master of Science in Clinical & Translational Research
In the Department of Environmental Health Division of Epidemiology of the College of Medicine
February, 2017

by

Tarek Alsaied, MD
M.D., University of Damascus, Faculty of Medicine, 3/25/2008
American Boards of Pediatrics, 10/25/2014
Committee Chair: Erin Haynes, DrPh
James F Cnota, MD
Eileen King, PhD
ABSTRACT:  
Background: Combined cardiac output (CCO) and cerebral autoregulation impact on fetal growth may vary in different congenital heart defects. This study compared serial measures of fetal growth, CCO, middle cerebral artery and umbilical artery pulsatility indices (PIs) as indicators for vascular resistance in four groups: hypoplastic left heart (HLHS), non-HLHS single ventricle (SV), transposition of great arteries (TGA) and normal controls.

Methods and results: Fetal echocardiograms from 109 fetuses were reviewed: HLHS (n=30), non-HLHS SV (n=20), TGA (n=17) and controls (n=42). CCO was calculated using valvar area, velocity time integral and heart rate. PIs were calculated using systolic, diastolic and mean velocities. Anthropometric measures were recorded. Regression models were used to study CCO, PIs and fetal anthropometric trends over gestational age. Multivariate analysis was used to determine the association of CCO and PIs at 30 weeks with birth weight, length and head circumference z-scores.

CCO increased in all 4 groups through gestation but plateaued in HLHS and SV at the end of gestation. Middle cerebral artery PI values were lower in HLHS compared to non-HLHS SV through gestation suggesting a different cerebral blood distribution. At the end of gestation, fetal weight plateaued in HLHS and SV
(similar to CCO curves) and head circumference plateaued in all groups but controls. CCO positively correlated with birth weight z-scores (p=0.003) and birth length z-scores (p=0.04). There was a trend toward a positive association between CCO and head circumference z-score (p=0.06). PIs did not correlate with any of the birth measurements.

**Conclusions:** CCO positively correlates with birth weight and may provide one mechanism to understand differences in fetal growth in congenital heart defects. Different brain autoregulation mechanisms are noticed between the groups. A brain sparing mechanism in HLHS is supported by lower cerebral vascular resistance.
ACKNOWLEDGMENTS:
I would like to thank the co-investigators and mentors on this project.

Eunice Hahn, MD\textsuperscript{a}, Stephanie Tseng ,MD\textsuperscript{b}, Eileen King, PhD\textsuperscript{c},  Allison Divanovic, MD\textsuperscript{a}, Mounira Habli, MD\textsuperscript{d}, James Cnota, MD\textsuperscript{a}.

\textsuperscript{a} Children’s Heart Institute, Cincinnati Children’s Hospital Medical Center, MLC 2003, 3333 Burnet ave, Cincinnati, OH, USA 45229.

\textsuperscript{b} Department of Pediatrics, Cincinnati Children’s Hospital Medical Center,  3333 Burnet ave, Cincinnati, OH, USA 45229.

\textsuperscript{c} Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, MLC 5041, 3333 Burnet Ave, Cincinnati, OH 45229, USA.

\textsuperscript{d} Division of Maternal Fetal Medicine, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229, USA.

Special thanks to Dr. Erin Haynes, PhD who was the advisor for this project and the chair of the committee.
TABLE OF CONTENTS

Abstract .......................................................................................................................... ii
Acknowledgments ........................................................................................................ v
List of tables figures ..................................................................................................... vi
Background .................................................................................................................. 1
Methods and Materials
    Study design .............................................................................................................. 2
    Fetal echocardiogram ............................................................................................... 3
    Statistical Analysis ................................................................................................... 3
Results
    Demographics .......................................................................................................... 4
    Fetal echocardiographic measurements .................................................................. 5
    Fetal growth .............................................................................................................. 6
Discussion ..................................................................................................................... 7
References .................................................................................................................... 12
Tables and Figures ....................................................................................................... 16
LIST OF TABLES AND FIGURES:

**Table 1**: Baseline maternal characteristics.

**Table 2**: Summary of the fetal information for fetuses in the 4 groups.

**Table 3**: Fetal indexed cardiac output and pulsatility indices z-score comparison.

**Table 4**: Fetal indexed cardiac output and pulsatility indices below and above 30 weeks of gestation.

**Table 5**: Birth anthropometrics and z-scores comparison between the groups.

**Figure1**: Combined cardiac output trend over gestation.

**Figure2**: Middle cerebral artery pulsatility index over gestation.

**Figure3**: Umbilical artery pulsatility index over gestation.

**Figure4**: Estimated fetal weight trend over gestation.

**Figure5**: Estimated head circumference trend over gestation.
Background

Congenital heart disease (CHD) is a leading cause of neonatal mortality and has significant long term impact on neurodevelopment.[1] Different types of CHD may have different fetal growth patterns.[1-3] Children with single ventricle physiology have lower birth weights and smaller head circumferences when compared to normal newborns. In contrast, children with transposition of the great arteries (TGA) have normal birth weights and slightly smaller head circumferences when compared to normal newborns.[1-3] Birth weight is a critical predictor for survival outcomes of congenital heart disease, especially in children with single ventricle (SV), and mortality is higher in patients with SV who are small for gestational age.[4-6] Head circumference at birth also affects the neurological outcomes of children with SV, and microcephaly increases the risk of neurodevelopmental delay in SV.[1, 7, 8]

These differences in fetal growth are likely multifactorial, but previous work suggests that variations in fetal circulation may affect fetal growth.[2] Recent studies of fetal cardiac MRI supported this theory and showed decreased combined cardiac output (CCO) in the late pregnancy by 20% in fetuses with hypoplastic left heart syndrome (HLHS) compared to normal fetuses and to fetuses with TGA.[9, 10] The effect of these findings on fetal growth were not studied.

Fetal echocardiography is a valuable tool which can be applied serially in clinical and research settings to evaluate fetal hemodynamics by estimating cardiac output and vascular resistance in the fetus.[11]

Fetal echocardiogram derived CCO has been validated in normal babies and normal values per gestational age have been established.[12] Clinically, CCO is used to evaluate the fetal high cardiac output lesions like arteriovenous malformations.[13] CCO has not been extensively studied in fetuses with congenital heart disease. One study showed that fetal CCO in HLHS is lower than normal fetuses by
Fetal echocardiography also provides important insight into fetal blood distribution using middle cerebral and umbilical artery pulsatility indices as markers of vascular resistance.[12, 15] Previous work showed decreased middle cerebral artery pulsatility index (MCA-PI) in HLHS and TGA.[16, 17] This observation was thought to reflect decreased cerebral vascular resistance to improve cerebral perfusion and oxygen delivery.[16, 17] In normal fetuses, fetal growth restriction is associated with decreased CCO and MCA-PI.[15]

The effects of fetal hemodynamics on fetal growth using fetal echocardiogram have not been studied in congenital heart disease.[15] The objective of this study was to evaluate fetal growth and the effect of fetal hemodynamics on fetal growth in different types of congenital heart disease including HLHS, non hypoplastic left heart syndrome single ventricle (non-HLHS SV) and TGA.

Materials and methods

Study Design:

This study is a retrospective multicenter cohort study and was approved by the IRB at Cincinnati Children’s Hospital Medical Center, University of Cincinnati Hospital and Good Samaritan Hospital in Cincinnati. We included all infants with a prenatal diagnosis of TGA, HLHS and non HLHS SV between January 1st, 2011 and March 1st, 2015, whose mothers underwent obstetric care at the University of Cincinnati and Good Samaritan Hospital, and fetal cardiology prenatal care at the Cincinnati Children's Hospital. The control group included infants with a structurally normal heart who underwent fetal echocardiography due to family history of congenital heart disease. The study population was divided into four groups: HLHS, non-HLHS SV, TGA and normal controls. We excluded subjects with known genetic diagnoses prenatally, major extra-cardiac anomalies, or the outcome of intrauterine death. Multiple gestation pregnancies were also excluded.
The obstetrical notes were reviewed from prenatal care visits. Maternal demographic characteristics were collected including race, ethnicity and insurance type. Prenatal information focusing on maternal factors that might affect fetal growth including height and weight, pregnancy-induced hypertension, gestational diabetes and smoking status were collected. Any other suspected prenatal diagnosis using maternal ultrasound was collected.

*Fetal growth*

Fetal biometric measurements were made by the original sonographer. Estimated fetal weight (EFW) and head circumference (HC) were collected from serial obstetric ultrasounds throughout gestation. Birth weight, length and HC were recorded from delivery reports. The z-scores for birth anthropometrics were calculated using previously published methods.[18]

*Fetal echocardiogram*

Fetal echocardiograms were performed by a trained sonographer with expertise in fetal echocardiography. Echocardiograms were performed in accordance with published standards.[19] All examinations were performed using either an Acuson Sequoia C512 ultrasound system or an Acuson S2000 ultrasound system (Siemens Medical Solutions USA, Inc., Malvern, PA, USA) with commercially available curvilinear transducers. Pulsed-wave Doppler evaluation of flow patterns in the middle cerebral and umbilical artery were recorded. Left and right cardiac outputs were calculated through pulsed-wave Doppler of the aortic and pulmonary valves, using the following equation: cross-sectional area of the semilunar valve \((3.14 \times \text{valve radius}^2)\) \(\times\) velocity time integral \(\times\) heart rate. CCO was obtained by adding the left and right cardiac output. The CCO was then indexed to the estimated fetal weight (indexed CCO) as previous studies suggested that indexed CCO does not change by gestational age.[12] Middle cerebral artery pulsatility index (MCA-PI) and umbilical artery pulsatility index (UA-PI) were calculated using
systolic, diastolic and mean velocities.[20] Z-scores for the MCA-PI and UA-PI were calculated using previously described methods.[11, 21]

**Statistical analysis**

Baseline maternal characteristics were summarized using frequencies and percentages for categorical variables, and mean +/- SD or median and interquartile range for continuous variables. Categorical variables were analyzed using the Fisher Exact test and continuous variables were analyzed using the Kruskal-Wallis nonparametric test. Pairwise comparisons among the four study groups were made using the Dwass, Steel, Critchlow-Fligner nonparametric multiple comparison p-value adjustment. Indexed CCO, MCA-PI z-scores, and UA-PI z-scores that were measured closest to 30 weeks were analyzed using an analysis of variance test with Tukey-Cramer multiple comparison p-value adjustment, where needed. Linear, quadratic and spline regression models were used to study the trend of CCO, MCA-PI, UA-PI and fetal anthropometrics across gestational ages for the four study groups accounting for repeated measures. P-values<0.05 were considered statistically significant. As we noticed that the variance in fetal weight and head circumference growth curves in the study groups became visible at 30 weeks of gestation, a multivariate analysis was used to determine the association of CCO and pulsatility indices measured close to 30 weeks with subsequent birth weight, length and head circumference z-scores.

**Results:**

Studies from 109 fetuses who met inclusion criteria and who had at least one fetal cardiology prenatal visit and birth anthropometrics were reviewed. The fetuses had a diagnosis of HLHS (n=30), non-HLHS SV (n=20), TGA (n=17) and normal controls (n= 42). In total, 221 fetal echocardiogram and 411 maternal ultrasounds were reviewed. We were able to calculate CCO in 188 of the echocardiograms (85%). In the
rest of the studies, the most common cause of missing cardiac output data was the absence of semilunar valve Doppler interrogation.

The maternal characteristics are detailed in Table 1. Overall there were less African American mothers in the TGA group. Mothers with HLHS and non-HLHS SV were more likely to have public insurance. There were no other differences in maternal characteristics among the groups.

Fetal characteristics and fetal cardiac anatomy are detailed in Table 2. There was no difference in fetal gender distribution among the groups. Of note, nearly half of the fetuses in non-HLHS SV group had tricuspid atresia with normally related great vessels followed by pulmonary atresia/intact ventricular septum.

**Fetal echocardiographic measurements:**

The fetal CCO increased throughout gestation in all groups (p<0.001 for all groups). Visual inspection of graph 1 shows that the TGA and normal control groups had a steeper slope of CCO increase compared to non-HLHS SV and HLHS groups. (Figure 1) The indexed CCO was higher for patients with TGA compared to non-HLHS SV group (504 ± 32 versus 396 ± 26; p=0.01). (Table 3) There was a trend towards a lower indexed CCO in the non-HLHS SV compared to normal controls (p= 0.05, table 3).

MCA-PI curves over gestation are shown in figure 2 for the four study groups. Visual inspection of the graph shows that the HLHS had lower MCA-PI compared to normal control and that non-HLHS SV had higher MCA-PI compared to normal controls. When z-scores were compared, HLHS had lower MCA-PI compared to non-HLHS SV group (-1.8 ± 0.2 versus -0.3 ± 0.4, p=0.006). There was no significant difference between TGA and normal controls although the curve showed a trend towards lower MCA-PI values in TGA at the end of gestation. (Figure 2)

UA-PI decreased through gestation in all groups. (Figure 3) There was no difference among the groups in UA-PI z-scores (0.1, table 1).
We then evaluated these measurements before and after 30 weeks of gestations. (Table 4) Before 30 weeks of gestation, indexed CCO was higher in the TGA group compared to HLHS (p=0.01) and non HLHS SV (p=0.01) while there was no difference in indexed CCO after 30 weeks of gestation among the groups (p=0.18). MCA-PI was higher in the non-HLHS SV group compared to HLHS (p=0.005) before 30 weeks, while after 30 weeks the non-HLHS SV group had higher MCA-PI compared to HLHS (p< 0.001) and TGA (p=0.04). After 30 weeks, UA-PI was higher in non-HLHS SV compared to TGA (p=0.04).

**Fetal growth:**

Fetal weight and fetal head circumference trends through gestation are shown in figure 4 and figure 5, respectively. As would be expected, all groups demonstrated an increase in their weight and head circumference (p<0.001 for all groups and both measures) through gestation. The graph shows decreased growth velocity in the non-HLHS SV and HLHS groups when compared to the TGA and normal groups. This difference was more pronounced at the end of gestation. For head circumference, visual inspection of figure 5 showed that HLHS, non-HLHS SV and TGA groups had decreased growth velocity compared to normal group. The difference was also more pronounced at the end of gestation.

Birth weight z-score was lower in non-HLHS SV compared to normal controls (p=0.04, table 5). Birth length z-score was lower in HLHS (p=0.02) and non-HLHS SV (p=0.006) compared to normal controls. Head circumference z-score was lower in non-HLHS SV compared to normal controls (p=0.003). Multivariate analysis showed a positive association between CCO and birth weight z-score (p=0.003) and CCO and birth length z-score (p=0.04). There was a trend toward a positive association between CCO and head circumference z-score (p=0.06). UA-PI and MCA-PI did not correlate with any of the birth measurements. Of note, there was a trend toward a negative association between UA-PI and birth length z-score (p=0.07).
Discussion:

This study demonstrates changes in cardiac output in different congenital heart disease lesions throughout gestation, and correlates these changes to fetal growth. Fetuses with single ventricle physiology demonstrate a more gradual cardiac output increase throughout gestation compared to patients with TGA and normal controls. There is a positive association between CCO and birth weight and birth length z-scores and a trend towards a positive association with head circumference z-score. This data may explain the variations in fetal growth patterns in different types of congenital heart disease.

Fetal hemodynamics by Doppler echocardiography

Figure 1 shows that fetuses with single ventricle physiology manifest lower CCO at the end of gestation. Previously, Szwast et al showed that CCO in fetuses with HLHS was lower by 20% as compared to normal fetuses. [14] Fetal cardiac MRI studies confirmed these findings in HLHS fetuses. [9, 22] This suggests that although the single ventricle compensates for the absence of other ventricular output, this compensation is incomplete and results in lower CCO in this patient population.

Our study also demonstrates a higher indexed CCO in TGA fetuses compared to other types of congenital heart disease. Interestingly, we found that the increase in indexed CCO in TGA is mainly below 30 weeks of gestation. Similarly, a study by Porayette et al using fetal cardiac MRI reported an increase in CCO in TGA. [10] The higher CCO in TGA is not well understood but may be explained by previous work studying fetal physiology of TGA. [23] In fetuses with TGA the left ventricular blood with high oxygen saturation flows to the pulmonary artery which leads to lower pulmonary vascular resistance. [23] This may result in a significant increase in pulmonary blood flow, and therefore, an overall increase in indexed CCO early in gestation. [24] As pregnancy progresses, the increased pulmonary blood flow results in elevated left atrial pressure which may lead to premature closure or restriction of blood flow at the level of the foramen ovale. In addition, higher pulmonary blood flow may
lead to less blood flow in the ductus arteriosus, which may also become restrictive later in gestation.[23] This explains the relative decrease in cardiac index later in gestation in this patient population.

Our study also investigated cerebral autoregulation in different congenital heart disease groups. Overall, the trend of MCA PI over gestation was similar to published trends, with a peak MCA PI around 30 weeks of gestation. [25] Our study demonstrated a higher MCA PI in non-HLHS single ventricle patients compared to HLHS patients. In HLHS, the lower MCA-PI suggests a vasodilatory response in the cerebral vasculature as there is retrograde flow through the transverse arch. The higher MCA-PI in non HLHS SV patients in our study suggests that there is a higher cerebral vascular resistance in this group. This can be explained by the fact that most of the fetuses in the non-HLHS SV group in our study have right ventricular outflow tract obstruction; thus, the majority of the CCO flows through the ascending aorta. The elevated cerebral vascular resistance suggests a brain regulatory mechanism to control cerebral blood flow in this population. These findings support previous work by Kaltman et al. and Szwast et al. which suggested that the fetal cerebral vascular resistance is regulated by the amount of blood flow in the non-HLHS SV group.[16, 26] We found no difference in MCA-PI between TGA and normal controls although there is a trend towards lower MCA-PI at the end of gestation in TGA. A previous study showed that the MCA-PI is decreased in TGA between 36-38 weeks of gestation compared to normal controls.[17] Recent fetal MRI studies suggested that the cerebral blood flow is preserved in patients with single ventricle physiology despite the significantly lower cardiac output, and that the lower oxygen delivery is likely due to lower aortic saturation.[22, 27] This supports the brain sparing mechanism previously reported in single ventricle patients, although it suggests that this mechanism is incomplete.[28-30]

**Fetal hemodynamics and fetal growth**

This study found different curves of weight gain for fetuses with single ventricle physiology as compared to fetuses with TGA and normal fetuses. Previous work has demonstrated that fetuses with HLHS have
decreased growth velocity later in gestation.[4] Hahn et al. showed that fetuses with single ventricle physiology have evidence of growth impairment later in gestation reflected by lower z-scores for estimated fetal weight as gestational age advances.[11] Similarly, many studies that evaluated estimated fetal weight with single ventricle physiology showed lower z-scores compared to normal controls.[31, 32] An understanding of these fetal growth patterns with single ventricle physiology may aid in the obstetrical clinical decision making while improving the efforts to prenatally risk stratify this patient population.

Our study suggests a positive association between fetal CCO around 30 weeks of gestation, and birth weight and length z-scores in addition to a trend towards a positive association with birth head circumference z-score. The theoretical relationship between fetal hemodynamics and fetal cardiac output in congenital heart disease was first observed in epidemiologic studies which showed that newborns with TGA are normal in size and larger than newborns with HLHS after adjusting for multiple other factors that may affect fetal growth.[2] We chose 30 weeks as differences in fetal growth became more pronounced at that gestational age. The potential implications of our findings warrant further evaluation. In particular, the temporal relationship between the drop in CCO and the fetal growth restriction needs to be investigated. Furthermore, whether the drop in CCO happens before growth restriction and may provide mechanistic insights about placental development abnormalities or whether this drop in CCO simply mirrors poor fetal weight gain is unclear.[33] These questions deserve further evaluation.

The fetal head circumference growth curves were also flattened at the end of gestation in the HLHS, non-HLHS SV and TGA groups. The multi-institutional study by Hahn et al. suggests a slowing in head circumference growth later in gestation in fetuses with single ventricle physiology.[11] A recent meta-analysis showed a pooled prenatal head circumference z-score of -0.51 in a mixed group of congenital heart disease. The findings can be extrapolated to single ventricle patients as these patients represented
the majority of fetuses in the included studies. [34] The smaller head circumference in congenital heart disease is likely due to smaller brain size attributed to decreased cerebral oxygen delivery. [22, 35, 36] Our study also suggested no association of the cerebral pulsatility indices with head circumference which may be explained by the differences in cerebral autoregulation mechanisms between the groups and the interaction between CCO and MCA-PI. It is possible that cerebral autoregulation mechanisms in patients with single ventricle physiology and TGA promote normal cerebral blood flow, although it is not able to maintain oxygen delivery and may result in a smaller brain size in these fetuses. [22, 27] Hahn et al. showed no association between MCA-PI or MCA-PI z-scores and head circumference at birth as well. [11] In addition to growth, these circulatory differences may adversely affect brain development and maturation. [37-40]

As understanding of fetal growth in congenital heart disease increases, most acknowledge that it is a complex process. [41] Although fetal hemodynamics may affect fetal growth, there are other genetic, placental and maternal factors that are likely extremely important determinants of fetal growth and the interaction between these factors and hemodynamics warrant further investigation. [33, 42, 43] Fetal cardiac and brain MRI imaging may add to our understanding when combined with fetal echocardiogram. [22, 27, 39]

**Limitations**

Our study has several limitations. The sample size was small in some of the groups (n=17 for TGA group). Also, this study excluded all patients with genetic diagnosis and major extra cardiac abnormalities which is a common contributing factor that results in impaired fetal growth. [43, 44] Fetal data were obtained retrospectively, and measurements could not, therefore, be standardized between sonographers. Study data were limited to those subjects who had fetal data available. Not all subjects had serial examinations, and not all measurements were available for every examination. CCO could not be
calculated in all the fetal echocardiograms depending on the image quality and the availability of Doppler tracings although we were able to calculate CCO in 85% of the patients. Normal subjects mostly had only a single fetal echocardiogram which was accounted for in the statistical models. Fetal echocardiograms and ultrasounds were done at wide range of prenatal ages. We acknowledge the importance of confirming these results in a larger sample size, in a prospective manner to determine whether the CCO is a potential cause or a result of fetal growth restriction.

Conclusion

Fetal echocardiography is an important tool to evaluate fetal hemodynamics in congenital heart disease. Our findings show a lower CCO trend in single ventricle patients towards the end of gestation, which correlated with lower birth weight and birth length. The cause-effect relationship of this observation will need to be further investigated and will be the focus of future studies. Correlating CCO with postnatal outcomes will help delineate the importance of CCO for fetal cardiovascular wellbeing in this patient population. The middle cerebral artery pulsatility index was significantly lower in the HLHS compared to the non-HLHS SV group, which did not correlate with fetal growth, but may reflect differences in fetal cerebral autoregulation mechanisms. Correlating these findings to neurodevelopmental outcomes will enhance the clinical usefulness of these measurements. Using fetal echocardiographic measurements may aid the efforts of risk stratifying patients with single ventricle physiology and potentially guide fetal therapies in this patient population.
References:


Table 1: Baseline maternal characteristics.

<table>
<thead>
<tr>
<th>Study group</th>
<th>HLHS (n=30)</th>
<th>Non HLHS SV (n=20)</th>
<th>TGA (n=17)</th>
<th>Normal (n=42)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity (Hispanic)</td>
<td>0</td>
<td>1 (5%)</td>
<td>0</td>
<td>2(5%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>4(13%)</td>
<td>5(25%)</td>
<td>0</td>
<td>9(21%)</td>
<td>0.03</td>
</tr>
<tr>
<td>White</td>
<td>25(84%)</td>
<td>13(65%)</td>
<td>16(94%)</td>
<td>24(58%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (3%)</td>
<td>2 (10%)</td>
<td>1 (6%)</td>
<td>9 (21%)</td>
<td></td>
</tr>
<tr>
<td>Public insurance</td>
<td>13 (43%)</td>
<td>9 (45%)</td>
<td>5 (29%)</td>
<td>11 (26%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>0 (0%)</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
<td>5 (12%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>164.9 ± 5.5</td>
<td>164.2±7.6</td>
<td>163.2±6.6</td>
<td>164.3±6.7</td>
<td>0.91</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>3 (10%)</td>
<td>1 (5%)</td>
<td>0</td>
<td>3 (7%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Maternal diabetes before pregnancy</td>
<td>1 (3%)</td>
<td>1(5%)</td>
<td>3 (18%)</td>
<td>1 (2%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>6 (20%)</td>
<td>0</td>
<td>1 (6%)</td>
<td>2 (5%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Parity</td>
<td>1.7 ± 0.8</td>
<td>3.1 ± 1.6</td>
<td>2.0 ± 1.23</td>
<td>2.2 ± 1.4</td>
<td>0.07</td>
</tr>
<tr>
<td>Maternal preeclampsia</td>
<td>0</td>
<td>1(mild)</td>
<td>1(mild)</td>
<td>1(severe)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 1: The results are presented as frequency (%) or mean± standard deviation. HLHS: Hypoplastic Left Heart Syndrome; Non HLHS SV: Non-hypoplastic left heart syndrome single ventricle; TGA: transposition of great arteries.
Table 2: Summary of the fetal information for fetuses in the 4 groups.

<table>
<thead>
<tr>
<th>Study group</th>
<th>HLHS (n=30)</th>
<th>Non HLHS SV (n=20)</th>
<th>TGA (n=17)</th>
<th>Normal (n=42)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra-cardiac diagnosis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Male gender</td>
<td>20 (67%)</td>
<td>13 (65%)</td>
<td>12 (71%)</td>
<td>28 (67%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Details of anatomy in fetuses with</td>
<td>MA/AA 18</td>
<td>MA/AA 6</td>
<td>TGA +VSD 5</td>
<td>TGA 12</td>
<td>NA</td>
</tr>
<tr>
<td>congenital heart disease</td>
<td>MS/AA 6</td>
<td>MS/AA 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS/AS 5</td>
<td>MS/AS 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS/AS and a small VSD</td>
<td>Unbalanced canal 4 (3 RV dominant and 1 LV dominant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VSD 1</td>
<td>Tricuspid atresia 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PA/IVS 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: AA: Aortic atresia; AS: Aortic stenosis; HLHS: Hypoplastic left heart syndrome; LV: Left ventricle; MA: Mitral atresia; MS: Mitral stenosis; NA: not applicable; Non HLHS SV: Non-hypoplastic left heart syndrome single ventricle; PA/IVS: Pulmonary atresia intact ventricular septum; RV: Right ventricle; VSD: Ventricular septal defect and TGA: transposition of great arteries.
Table 3: Fetal indexed cardiac output and pulsatility indices z-score comparison.

<table>
<thead>
<tr>
<th>Group</th>
<th>Indexed CCO (ml/kg/min)</th>
<th>Pairwise comparisons</th>
<th>MCA-PI z-score</th>
<th>Significant pairwise comparisons</th>
<th>UA-PI z-score</th>
<th>Significant pairwise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLHS (n=30)</td>
<td>422 ± 21</td>
<td>0.01 (TGA versus Non HLHS SV)</td>
<td>-1.8 ± 0.2</td>
<td>0.006 (Non HLHS SV versus HLHS)</td>
<td>0.1 ± 0.2</td>
<td>0.08 (Non HLHS SV vs TGA) P &gt; 0.1 for other comparisons</td>
</tr>
<tr>
<td>Non HLHS SV (n=20)</td>
<td>389 ± 22</td>
<td>0.05 (normal versus Non HLHS SV)</td>
<td>-0.3 ± 0.5</td>
<td>0.07 (normal versus Non HLHS SV)</td>
<td>0.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>TGA (n=17)</td>
<td>505 ± 30</td>
<td>0.1 (TGA vs HLHS) P &gt; 0.1 for other comparisons</td>
<td>-0.7 ± 0.4</td>
<td>0.08 (HLHS vs TGA)</td>
<td>-0.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Normal(n=42)</td>
<td>496 ± 22</td>
<td></td>
<td>-1.3 ± 0.2</td>
<td></td>
<td>-0.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The results are least square means ± standard error of the mean. CCO: combined cardiac output; HLHS: Hypoplastic left heart syndrome; MCA-PI: Middle cerebral artery pulsatility index; Non HLHS SV: Non-hypoplastic left heart syndrome single ventricle; TGA: transposition of great arteries; UA-PI: Umbilical artery pulsatility index.
Table 4: Fetal indexed cardiac output and pulsatility indices below and above 30 weeks of gestation.

<table>
<thead>
<tr>
<th>Gestational age group</th>
<th>Study group</th>
<th>Indexed CCO (ml/kg/min)</th>
<th>Significant pairwise comparisons</th>
<th>MCA-PI</th>
<th>Significant pairwise comparisons</th>
<th>UA-PI</th>
<th>Significant pairwise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before 30 weeks of gestation</strong></td>
<td>HLHS</td>
<td>440 ± 31</td>
<td>0.01 (TGA versus HLHS)</td>
<td>1.40 ± 0.06</td>
<td>0.005 (Non HLHS SV versus HLHS)</td>
<td>1.17 ± 0.04</td>
<td>P &gt; 0.1 for all comparisons</td>
</tr>
<tr>
<td></td>
<td>Non HLHS SV</td>
<td>433 ± 29</td>
<td>0.01 (TGA versus Non HLHS SV) P &gt; 0.1 for other comparisons</td>
<td>1.74 ± 0.07</td>
<td>1.24 ± 0.04</td>
<td>1.14 ± 0.06</td>
<td>P &gt; 0.1 for other comparisons</td>
</tr>
<tr>
<td></td>
<td>TGA</td>
<td>610 ± 47</td>
<td>P &gt; 0.1 for other comparisons</td>
<td>1.69 ± 0.11</td>
<td>1.55 ± 0.07</td>
<td>1.31 ± 0.04</td>
<td>P &gt; 0.1 for other comparisons</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>501 ± 27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>After 30 weeks of gestation</strong></td>
<td>HLHS</td>
<td>409 ± 25</td>
<td>P &gt; 0.1 for all comparisons</td>
<td>1.43 ± 0.06</td>
<td>&lt;.0001 (Non HLHS SV versus HLHS)</td>
<td>1.03 ± 0.04</td>
<td>0.04 (Non HLHS SV versus TGA) P &gt; 0.1 for other comparisons</td>
</tr>
<tr>
<td></td>
<td>Non HLHS SV</td>
<td>343 ± 29</td>
<td></td>
<td>1.89 ± 0.07</td>
<td>1.12 ± 0.05</td>
<td>0.92 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGA</td>
<td>438 ± 37</td>
<td></td>
<td>1.59 ± 0.09</td>
<td>0.92 ± 0.06</td>
<td>1.00 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>412 ± 35</td>
<td></td>
<td>1.62 ± 0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: The results are least square means ± standard error of the mean. CCO: combined cardiac output; HLHS: Hypoplastic left heart syndrome; MCA-PI: Middle cerebral artery pulsatility index; Non HLHS SV: Non-hypoplastic left heart syndrome single ventricle; TGA: transposition of great arteries; UA-PI: Umbilical artery pulsatility index.
Table 5: Birth anthropometrics and z-scores comparison between the groups.

<table>
<thead>
<tr>
<th>Study group</th>
<th>HLHS (n=30)</th>
<th>Non HLHS SV (n=21)</th>
<th>TGA (n=17)</th>
<th>Normal (n=42)</th>
<th>Significant pairwise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth weight (gram)</strong></td>
<td>3084 ± 542</td>
<td>2773 ± 463</td>
<td>3151 ± 413</td>
<td>3316 ± 581</td>
<td>0.002 (Non HLHS SV vs normal)</td>
</tr>
<tr>
<td><strong>Birth weight z-score</strong></td>
<td>-0.5 ± 0.9</td>
<td>-0.6 ± 0.6</td>
<td>-0.2 ± 0.7</td>
<td>-0.1 ± 0.9</td>
<td>0.04 (Non HLHS SV vs. normal)</td>
</tr>
<tr>
<td><strong>Birth length (cm)</strong></td>
<td>49 ± 2.7</td>
<td>47.4 ± 3.4</td>
<td>49.0 ± 1.9</td>
<td>50.7 ± 2.7</td>
<td>0.02 (HLHS vs normal)</td>
</tr>
<tr>
<td><strong>Birth length z-score</strong></td>
<td>-0.5 ± 0.8</td>
<td>-0.7 ± 0.8</td>
<td>-0.4 ± 0.6</td>
<td>0.1 ± 0.9</td>
<td>0.02 (HLHS vs. normal)</td>
</tr>
<tr>
<td><strong>Birth head circumference (cm)</strong></td>
<td>33.5 ± 2.2</td>
<td>32.1 ± 2.2</td>
<td>33.5 ± 0.7</td>
<td>34.4 ± 1.8</td>
<td>0.0009 (Non HLHS vs normal)</td>
</tr>
<tr>
<td><strong>Head circumference z-score</strong></td>
<td>-0.4 ± 1.2</td>
<td>-0.9 ± 1.1</td>
<td>0.3 ± 0.6</td>
<td>0.2 ± 1.0</td>
<td>0.003 (Non HLHS vs. normal)</td>
</tr>
<tr>
<td><strong>Gestational age at birth (week)</strong></td>
<td>38.6 ± 1.4</td>
<td>37.6 ± 2.2</td>
<td>38.1 ± 1.4</td>
<td>38.9 ± 1.8</td>
<td>0.02 (Non HLHS vs. normal)</td>
</tr>
</tbody>
</table>

Table 5: The results are means ± standard deviation. All the comparisons not mentioned in the table had a p-value >0.1. HLHS: Hypoplastic left heart syndrome; MCA-PI: Middle cerebral artery pulsatility index; Non HLHS SV: Non-hypoplastic left heart syndrome single ventricle; TGA: transposition of great arteries; UA-PI: Umbilical artery pulsatility index.
Figure1: Combined cardiac output trend over gestation. The hypoplastic left heart syndrome and the non HLHS single ventricle groups has a more gradual increase in CCO compared to normal controls and transposition of the great arteries. HLHS: Hypoplastic left heart syndrome.
Figure 2: Middle cerebral artery pulsatility index over gestation. The hypoplastic left heart syndrome group had lower MCA-PI while non HLHS single ventricle group had higher MCA-PI compared to normal controls. All the 4 groups had a peak MCA-PI around 30 weeks of gestation. The transposition of the great arteries group had a trend to low MCA-PI compared to normal at the end of gestation. HLHS: Hypoplastic left heart syndrome.
**Figure 3**: Umbilical artery pulsatility index over gestation. All the groups have a decrease in UA-PI over gestation with no statistically significant difference in the slopes. HLHS: Hypoplastic left heart syndrome.
Figure 4: Estimated fetal weight trend over gestation. The hypoplastic left heart syndrome and the non HLHS single ventricle groups has a more gradual fetal weight increase compared to normal controls and transposition of the great arteries. HLHS: Hypoplastic left heart syndrome.
Figure 5: Estimated head circumference trend over gestation. The hypoplastic left heart syndrome, non HLHS single ventricle and the transposition of the great arteries group had a flattened curves for head circumference increase over gestation. HLHS: Hypoplastic left heart syndrome.