**BS14** IMAGE-BASED COMPUTATIONAL SIMULATIONS OF FOETAL HEART FUNCTION TO UNDERSTAND CONGENITAL MALFORMATIONS AND FOETAL HEART INTERVENTION

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Background Some Congenital Heart Malformations develops because of cardiac abnormalities during mid-gestation, which prevents normal development for the rest of gestation to lead to the malformation at birth. An example is foetal critical aortic stenosis, where an outflow obstruction causes high left ventricle (LV) pressures, low myocardial strains, and severe mitral regurgitation (MR). These abnormal conditions cause hypoplastic left heart Syndrome (HLHS) by birth in most cases, and are thus evolving HLHS cases. In such cases, catheter-based foetal aortic balloon valvuloplasty in utero interventions was shown to be promising in relieving the abnormal biomechanics, significantly reducing chances of single ventricular birth. However, the biomechanical nature of stenosis and intervention remain poorly characterized, even though they both have significant biomechanical effects. Further, our ability to predict outcomes of disease or intervention is very limited. We hypothesize that advanced image-based biomechanical simulations can improve our understanding, and can be used as a tool to better predict intervention outcomes. Here, we present preliminary work towards testing this hypothesis.

Methods 4D echocardiography images of foetal hearts from both healthy and diseased (critical aortic stenosis) foetuses were analysed for numerical reconstruction of the LV and its motion, using validated motion tracking algorithms. Image-based patient specific Finite Element Modeling of the LV was conducted to determine the biomechanical effects of various individual features of foetal aortic stenosis. This model featured both active tension and passive stiffness of myocardium, spatially varying myofiber orientations, and a simplified Windkessel model to describe ventricular-vascular coupling. Image-based computational fluid dynamics modeling of the LV was also conducted to understand flow patterns and forces in the LV during disease, compared to healthy hearts.

Results Fetal aortic stenosis alone was found to elevate LV pressures by 10-20 mmHg, and could drastically decrease stroke volume and myocardial strain, depending on severity. These effects were moderated down by MR. Our modelling indicated that stenosis alone could not lead to regurgitation velocities as high as clinical observations, unless hypertrophic wall thickening occurred. Indeed, our clinical data showed approximately 105 ± 37% wall thickening. Modelling further indicated that this typical extent of hypertrophy produced LV pressures much higher than clinical invasive measurements, suggesting that contractility also weakened. Fibroelastosis was tested in our model by increasing myocardial stiffness, but was found to be inconsequential to cardiac biomechanics and function, suggesting that the conventional belief that fibroelastosis causes dysfunction is not true, and that it could merely be a by-product of the disease. Flow Simulations showed that a fetal aortic stenosis caused fast and narrow inflow fluid jet that collided with the apex, and led to chaotic vorticity patterns in the LV, altered wall shear stresses patterns, and drastically increased energy losses and cardiac work done.

Conclusion We developed image-based simulation tools that can analyse the biomechanics and function of the foetal heart computationally. These simulations were able to provide insights into the disease conditions, and could thus be useful tools. Our future work is to use these simulations to predict the outcome of the foetal heart interventions.

Conflict of Interest None

**BS15** USING ZEBRAFISH EMBRYOS TO IDENTIFY GENES THAT REGULATE ENDOTHELIAL PROLIFERATION

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Introduction Atherosclerosis describes the formation of plaques in the arteries which can lead to myocardial infarction and stroke. Atherosclerosis preferentially occurs in regions of the arteries where the endothelium is exposed to low shear stress, in part, because endothelium in these regions undergoes excessive proliferation. Understanding the mechanisms for this could allow identification of therapeutic targets for patients at risk of cardiovascular disease. We hypothesise that zebrafish embryos can be used to analyse endothelial proliferative responses to flow. If substantiated, this model has the potential to replace rodent models of endothelial pathology.

Methods Candidate regulators of endothelial cell (EC) proliferation in response to flow were identified in prior work (Serbanovic-Canic et al, 2017).

EC proliferation was quantified by visualising nuclei of Tg fli1a:nls-mCherry embryos in the intersegmental vessels (ISVs) and dorsal longitudinal anastomotic vessel (DLAV) between 54 and 72 hours post fertilisation using a Zeiss LSM880 microscope.

Microinjection of morpholino antisense oligonucleotides into single-cell embryos was used to knock-down genes of interest, non-targeting control morpholino and uninjected embryos were used as controls. Flow was prevented by morpholino knockdown of tnt2a.

Results Genes that are putative regulators of EC proliferation in response to flow were identified by mining microarray data generated from low and high shear stress regions of the aorta (Serbanovic-Canic et al, 2017). Expression of these genes in zebrafish was validated by quantitative RT-PCR analysis of zebrafish endothelial cells isolated by FACs of cells expressing fli1-EGFP. Morpholinos were then designed against each of these genes to identify those that regulate EC proliferation in response to flow.

Endothelial cell proliferation was quantified in embryos with and without blood flow, proliferation was significantly reduced in no-flow embryos (0.81% vs 0.26%; P=0.43). Knockdown of wnk1a significantly increases the rate of EC proliferation in no-flow embryos (0.26% vs 0.69%; P=0.014), whereas knockdown of gsk3b and fzd5 significantly decreases the rate of EC proliferation in embryos with blood flow (gsk3b 0.51% vs 0.81%; P=0.013. fzd5 0.46% vs 0.81%; P=0.002).

Conclusions Endothelial cell proliferation is reduced in the absence of flow in zebrafish. Putative regulators of EC
proliferation in response to flow were identified. Future work will validate the role of these genes in endothelial proliferation and atherosclerosis using mammalian models, and human cells and tissues.

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REFERENCE

Conflict of Interest none

BS16 THE ROLE OF PARASYMPATHETIC NERVOUS SYSTEM IN THE INFARCT-LIMITING EFFECT OF SGLT2 INHIBITORS
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Abstract BS16 Figure 1 The effect of parasympathetic denervation on acute cardioprotection by SGLT2 inhibitor Ertugliflozin (Ertu). IS – infarct size, AAR – area at risk. ** - p<0.01, *** - p<0.001.

BS17 RNA BINDING PROTEIN MULTIPLE SPlicing (RBPMS) DRIVES A CONTRACTILE SPlicing NETWORK IN HUMAN EMBRYONIC STEM CELL DERIVED VASCULAR SMOOTH MUSCLE CELLS
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Introduction Contractile Vascular Smooth Muscle cells (VSMCs) lining the walls of major arteries express a unique network of alternative splicing (AS) patterns in critical VSMC regulatory and functional genes (SM-AS). The regulation and impact of SM-AS on VSMC phenotype and behaviour, however, remain poorly understood. Previously, we uncovered a master splicing regulator, RNA Binding Protein Multiple Splicing (RBPMS), a factor highly expressed in tissue VSMCs. RBPMS contributes to about 20% of SM-AS seen in contractile VSMCs.1 2

Aim To understand the contribution of RBPMS and the SM-AS splicing network to VSMC function.

Basic Methods We have used human embryonic stem cell derived VSMCs (hES-VSMC)3 – a system compatible with stable genetic manipulation and facile molecular and phenotypic assessment. We generated hESC clones housing Doxycycline-inducible4 RBPMS alongside GFP at the pUCAAVS genomic safe harbour. Notably, induction from this locus was heterogeneous with GFP intensity acting as an indicator of RBPMS expression. This uniquely lends itself to downstream SM-AS and phenotypic comparison of cells with varying levels of RBPMS expression within the same population. For SM-AS assessment, mRNA-seq was performed on flow assisted cytometric sorted hES-VSMCs based on GFP expression followed by splicing network analyses using rMATS5, Matt6 and gene ontology (stringDB). For phenotype assessment, mitogen-induced proliferation of GFP high and low cells was monitored by estimating a cell-tracer dye dilution using flow cytometry (figure 2A). Motility was examined using live cell imaging followed by cell tracking analysis over 24 hours (ibidi) (figure 2B).

Results and Conclusions We observe that RBPMS-high cells possessed distinct splicing profiles from RBPMS-low cells. Statistical comparison of the two groups using rMATS5, revealed