The placenta in fetal congenital heart disease

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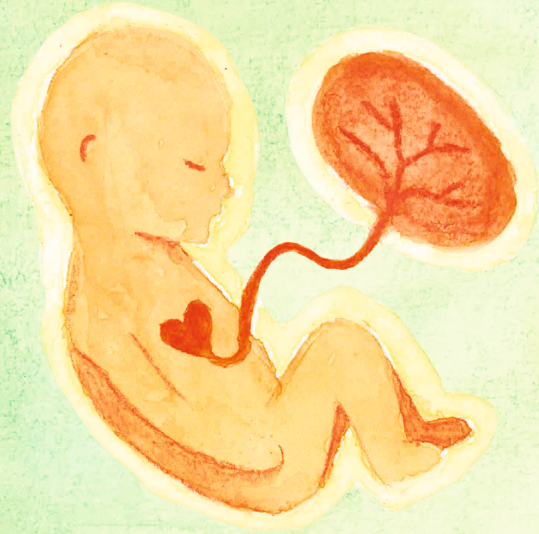

CHAPTER 9

Study set up
Placental characteristics of fetal lambs with induced decreased left ventricular output

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ABSTRACT

Fetal congenital heart defects (CHD) are associated with placental changes and placenta-related complications of pregnancy, such as pre-eclampsia and fetal growth restriction. How placenta formation and heart development interact, is still poorly understood and hardly studied. It is hypothesized that changes in the fetal circulation, as a result of the anatomy of the heart defect, could lead to altered placenta perfusion and result in abnormal placenta development.

In this study, a structured system to assess placenta histology in a sheep model is proposed. In this model, we aim to induce hypoplastic heart syndrome (HLHS) in an healthy lamb, with a healthy placenta development until intervention. Histological placental characteristics of intervention lambs will be assessed and compared to the placenta of healthy control siblings and placentas of lambs in which the intervention was attempted, but HLHS was not achieved (sham controls).

We hypothesize that abnormal placental development occurs in HLHS lambs, and that obstruction of left ventricular outflow contributes to these placental alterations. If the placentas of the intervention lambs are completely normal and comparable to the healthy controls or even siblings, the hypothesis is that the altered placenta development and fetal hypoxia found in human fetuses with CHD has another origin in development. That would steer hypotheses towards a process that changes cardiomorphogenesis and placenta development simultaneously at very early stages of embryology.

INTRODUCTION

It is known that 40% of the children with congenital heart disease (CHD) exhibit learning problems or developmental delays at school age [1-3]. It is assumed that a reduced oxygen supply to the brain in certain CHDs as early as in fetal life influences the development of the brain, based on the observation that human fetal brains in CHD show a delay in maturation [4, 5].

The placenta is a highly vascularized organ which is crucial for fetal growth and development. Genes involved in cardiogenesis and vascular development overlap and could therefor influence heart anatomy and placenta morphology [6, 7]. For this reason, interaction between the development of the placenta and the fetal heart is plausible and this is supported by the found histological placenta abnormalities in fetuses with CHD [8, 9]. Another supporting finding for this hypothesis is the increased incidence of placenta-related disorders, such as pre-eclampsia and fetal growth retardation, are demonstrated in pregnancies with fetal CHD [10-12].

How the interaction between placenta and heart development occurs is still unclear. On one hand, it is thought that altered placenta development in the very young embryo causes fetal hypoxia, which contributes to the development of a heart defect. Conversely, the developmental defect in the heart anatomy of the embryo, and with that the altered circulation due to the developing heart defect, could lead to reduced placenta perfusion, which could result in abnormal placenta histology and function [9, 13]. By further investigating the parallel development of congenital heart defects and placenta abnormalities, this might contribute to answering the question of why congenital heart defects arise.

Recent research by our group in which cerebral maturation in CHD fetuses was studied, did not demonstrate a relationship between brain development and aortic flow or oxygenation [5, 14-17]. Equal delay in brain maturation was found, independent of the type of heart defect, even in normal aortic flow and oxygenation. Yet, a much stronger relationship between fetal growth and the delayed brain maturation of fetuses with CHD was found.

Animal models may significantly help to address these knowledge gaps, particularly when specific experimental conditions can be controlled. This experimental setup aims to address the research question of whether the placenta contributes to heart and brain development, or whether cardiac defects lead to abnormal placental development and delayed brain maturation. An ideal model, developed by an international consortium, is one in which it is attempted to induced HLHS in an healthy lamb (with a healthy placenta) [18]. The primary and original goal of the research group is to investigate the effect of the induced heart defect on the flow and the development of the brain of the lamb, by MRI.

The aim of this ancillary study, conducted alongside the main project, is to examine the placentas of this animal model, to test the hypothesis that an abnormal placenta development will be observed in the intervention lambs. This would be a strong indicator that flow abnormalities in the left heart cause changes in the placenta. If the placenta of the intervention lambs is normal, the placenta findings of human fetuses with CHD must be explained by earlier developmental events that effect common development of the fetal heart and the placenta in earlier embryonic life.

In this study set up, we propose a structured system to examine sheep placentas, aiming to assess histological placental characteristics of intervention (HLHS) lambs and compare them to those of healthy control lambs.

MATERIALS AND METHODS

Coil implantation

The methods on coil implantation in the fetal lambs in this model have previously been described[18]. In short, a needle is percutaneously inserted in the fetal left atrium under continuous ultrasound guidance. One to 3 coils are advanced through this needle, a technique that has been adapted from the technique used for human fetal cardiac interventions. The coil is intended to be positioned within the annulus of the mitral valve, thereby mimicking mitral stenosis, which restricts inflow into the left ventricle and consequently results in hypoplastic left heart development. This is done at +/- 0.52 gestation (midway). As this is a challenging technique, and not all coils reach the right position, migrating to the roof of the left atrium and as a result not all fetal lambs that undergo the implantation evolve to HLHS. These cases are perfect 'sham' cases, being a good 'normal control', having a intervention, but not a HLHS. Twin lambs in the same mother sheep that do not undergo the procedure serve as unoperated controls.

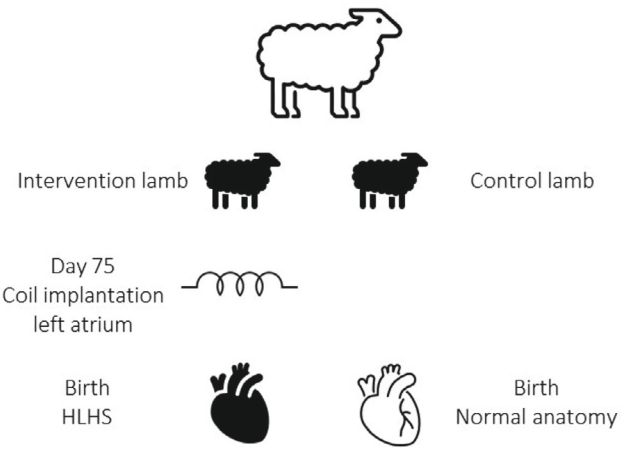

Figure 1: Animal model

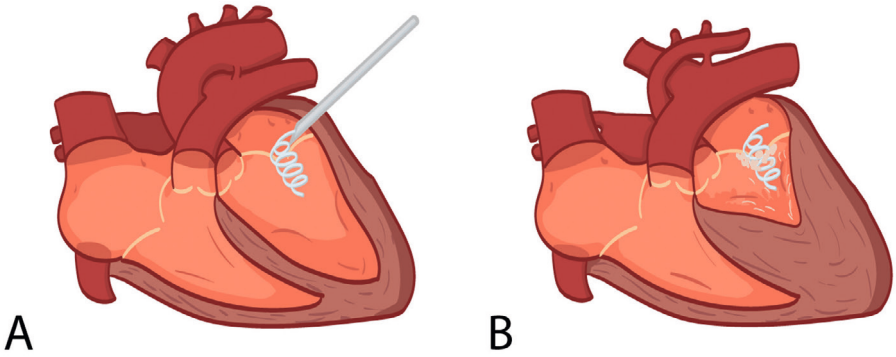

Figure 2: Schematic drawing of the coil intervention and expected outcome
A: coil implantation. B: development of HLHS. © Nina Odgaard

This animal model offers a unique opportunity to investigate and compare the placentas of the HLHS lambs with those of healthy lambs, often under the unique condition of identical circumstances in twin pregnancies.

Fetal lambs will be delivered by hysterotomy under maternal general anesthesia at 0.84 gestational age. The uterus is opened and the lamb will be delivered, after which a hysterectomy will be performed to collect the placenta and uterine wall.

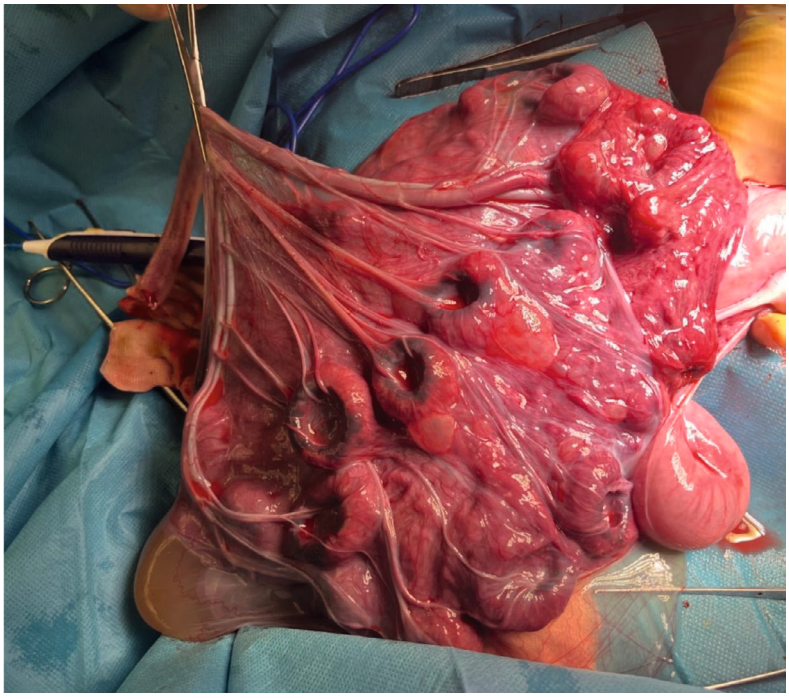

Figure 3: Macroscopic sheep placenta with loose, round cotyledons

Histology

Placentas will be collected by hysterectomy after delivery. The uterus with the placenta will be stored in 4% formaldehyde. Four cotelydones will be selected (two central, two peripheral) and membranes/chorionic plate and parenchyma will be selected. Standard clinical procedure for tissue processing will be used: after fixation in 4% formaldehyde, tissue samples were embedded in paraffin. Serial sections (4-µm thick) will be dried overnight at 37°C. After staining with hematoxylin and eosin (H&E), slides will be microscopically examined by an experienced perinatal pathologist (LM) and researcher (MS), who will be blinded for the group information and clinical data.

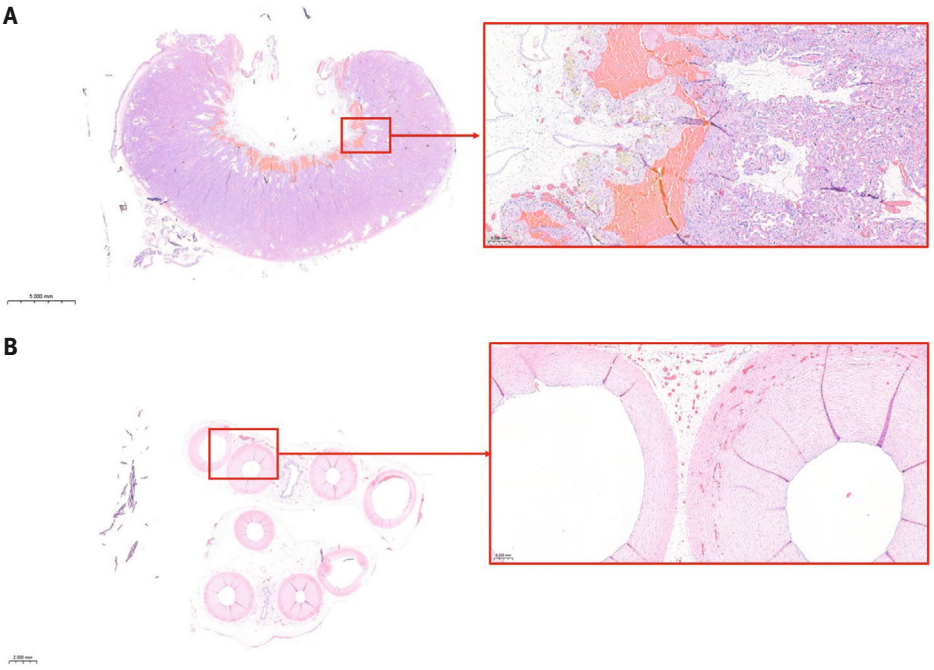

Figure 4: Histology of sheep placenta
Figure 4a: Cotelydon
Cross section of parenchyma with the fetal side (upper side, red) and the maternal side (lower side, purple). The parenchyma is build up of long vili surrounded with maternal blood.
Figure 4b: Umbilical cord
The umbilical cord has more vessels (arteries and veins) with an elastic vessel walls, surrounded by Whartons jelly.

We have altered the scoring system for human placentas [19, 20] into a simplified system for the assessment sheep placenta. The following characteristics can be scored: maternal vascular malperfusion (placenta ischemia, placental infarction), fetal vascular malperfusion (thrombosis) and inflammation or reactive changes (villitis, intervillositis, perivillous fibrin).

Table 1: Histology assessment of sheep placentas

	Yes/no
Maternal vascular malperfusion	
Ischemia	
Infarction	
Fetal vascular malperfusion	
Thrombosis	
Inflammation or reactive changes	
Villitis of unknown origin	
Intervillositis	
Perivillous fibrin	

Ethics

All procedures follow the Swiss ethical regulations for animal testing and were approved by the University of Zürich, committee for ethics of animal research (35180/ZH151/2022).

PRELIMINARY RESULTS

The development of a sheep placenta scoring system

To gain an initial understanding of the histological characteristics of sheep placentas, we examined three placentas of healthy unoperated lambs. Two were lambs in which a coil was inserted but moved to the roof of the right atrium, not resulting in HLHS, and one was a control in which a coil was placed in the sibling after which the sibling died.

Though sheep placenta placentas are structurally different from human placentas [21], we found that sheep placentas exhibit significant histological similarities to human placentas. Distinct maternal and fetal sides can be identified and the structural organization, including chorionic vessels, decidua, and parenchyma, are comparable. Interestingly, sheep placentas possess loose, round cotyledons (Figure 3), and their parenchyma is elongated rather than rounded as in humans (Figure 4a). The primary histological placental abnormalities, namely ischemia and inflammation, appear to be easily recognizable in sheep placentas, which is promising for our research.

FUTURE PERSPECTIVES

The parallel development of the fetal heart and the placenta and its possible interaction in congenital heart defects needs further investigation, to obtain more information about the origin of CHD. The animal model described in this study set up offers a unique opportunity to investigate and compare the placentas of the induced-HLHS lambs with those of healthy lambs, which is a great addition to current research on human CHD placentas. We found that sheep placentas exhibit significant histological similarities to

human placentas. Primary histological placental abnormalities, such as ischemia and inflammation, appear to be easily recognizable in sheep placentas. With the system to examine sheep placentas, proposed in this study set up, we aim to assess histological placental characteristics of intervention (HLHS) lambs and compare them to those of healthy control lambs in order to assess the effect of hemodynamic alterations induced by a cardiac defect on the placental development.

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