Feasibility of Phase-Contrast Cine MRI for Measuring Blood Flow in the Sheep Fetus

by

An Qi Duan

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

© Copyright by An Qi Duan 2017
Feasibility of Phase-Contrast Cine MRI for Measuring Blood Flow in the Sheep Fetus

An Qi Duan

Master of Science
Institute of Medical Science
University of Toronto
2017

Abstract

Phase-contrast cine magnetic resonance imaging (PC-MRI) is the gold-standard non-invasive technique for measuring vessel blood flow and has been applied in the human fetal circulation. We aimed to examine the feasibility of using PC-MRI to define the distribution of the fetal circulation in late gestation sheep. 13 fetuses underwent MRI at ~123 days gestation under isoflurane sedation, with the mother ventilated at an FiO2 of 1. PC-MRI was performed with blood pressure triggers from fetal arterial catheters. Blood flows were measured in major fetal vessels and indexed to estimated fetal weight. The mean flows were successfully obtained in 82% of the vessels targeted. Failed measurements resulted from poor visualization of target vessels. There was good interobserver agreement ($R^2=0.993$, $P<0.001$; ICC=0.996) and reproducibility in repeated acquisitions ($R^2=0.978$, $P<0.001$; ICC=0.990). Blood flow measurements showed variations from previous findings, likely due to pulmonary vasodilation in response to the increased fetal blood oxygen content we induced with maternal hyperoxygenation.
Acknowledgments

First and foremost, I would like to express my sincere gratitude to my graduate supervisor, Dr. Mike Seed, for his wholehearted guidance and generous support throughout my study period. Thank you for giving me the opportunity to work as part of your team. Your patience and insights helped me tremendously with my research. Your support for my future career has also been invaluable to me. Thank you so much.

I would like to thank my Program Advisory Committee members, Dr. Christopher Macgowan and Dr. Brian McCrindle. Thank you for dedicating your time and effort into guiding me through my study. I would also like to acknowledge the enormous contribution of all members of the Early Origins of Adult Health Research Group at the University of South Australia, including Professor Janna Morrison, Jack Darby, Mitchell Lock, Jia Yin Soo, Sunthara Rajan Perumal, and Lucy Flynn, who contributed their time and effort to the study. Without Professor Janna Morrison’s expertise, this study would not have been possible.

I also gratefully acknowledge the support of my lab mates, Dr. Seed’s research team: Jessie Mei Lim, Brahmdeep Saini, Meng Yuan Zhu, Dr. Davide Marini, Amandeep Saini, and Dr. Prashob Porayette, for providing me with care, encouragement, and assistance. I love you all. A sincere “thank you” to Sharon Portnoy and Elaine Stirrat, who have provided me with guidance and assistance through their expertise in MRI and animal studies.

I thankfully acknowledge the support of the Canada Graduate Scholarship, Hospital for Sick Children, the Institute of Medical Science, University of Toronto, and the course instructors, students and staff who have taught or worked with me.

Last but not least, a sincere “thankyou” to my parents and my loved one who have provided me with unconditional love and a place to call home. Thank you for making me who I am today, for enduring the worst of me, for always believing in me, and for encouraging me to pursue the life I want. Thank you.
Contributions

The Toronto supervisor, Mike Seed, his co-investigator, Chris Macgowan, and University of South Australia co-investigator, Janna Morrison, have an established research collaboration. Mike Seed and Janna Morrison conceived the experiment, which was conducted by them with assistance from Professor Morrison’s research team, the Early Origins of Adult Health Research Group, in Adelaide, Australia. The contribution of each individual is as follows:

The author, An Qi Duan, participated in the MRI scans, set up and monitored gating triggers for the MRI, and performed post-processing of the MR images and data collection and analysis.

Dr. Mike Seed and Sunthara Rajan Perumal performed the MRI scans on the sheep fetuses.

Led by Professor Janna Morrison, Jack R Darby, Jia Yin Soo, Mitchell C Lock, Dr Erin McGillick, Lucy Flynn, Katherine Stevens and Stacey Holman performed all animal work. This included planning for the timing of mating the ewes, transport to the animal facility, preparation for fetal surgery, fetal surgery, care of the ewe and her fetus for two weeks after surgery, collection of blood samples from the fetus, blood gas analysis, calibration and set up for monitoring fetal heart rate during the MRI scans and collection of fetal tissues in post mortem.

Meng Yuan Zhu performed interobserver variation measurements.

Lucy Flynn and Professor Janna Morrison analyzed blood pressure and heart rate record from MRI scans.

Dr. Christopher K Macgown and Dr. Joseph B Selvanayagam provided guidance on developing and understanding the MRI protocol and data collected.
# Table of Contents

Acknowledgments ......................................................................................................................... iii

Contributions ................................................................................................................................. iv

Table of Contents............................................................................................................................... v

List of Tables ........................................................................................................................................ viii

List of Figures ...................................................................................................................................... ix

List of Abbreviations ......................................................................................................................... xi

1 Introduction........................................................................................................................................... 1

1.1 Rationale.......................................................................................................................................... 1

1.1.1 The Importance of Monitoring Blood Flow .................................................................................... 1

1.1.2 Potential Methods for Measuring Blood Flow .............................................................................. 3

1.2 PC-MRI ........................................................................................................................................... 4

1.2.1 Basics of PC-MRI........................................................................................................................ 4

1.2.2 Fetal MRI and Cardiac Gating....................................................................................................... 8

1.2.3 Accuracy of PC-MRI Flow Measurements .................................................................................. 11

1.2.4 Potential Sources of Errors in PC-MRI Measurements ............................................................... 12

1.3 Other Methods of Measuring Blood Flow ..................................................................................... 15

1.3.1 Flow Probes ............................................................................................................................... 16

1.3.2 Labeled Microspheres .............................................................................................................. 18

1.3.3 Doppler Ultrasound ................................................................................................................ 20

1.4 Fetal Circulation ............................................................................................................................ 25

1.4.1 Postnatal Circulation ................................................................................................................. 25

1.4.2 Fetal Circulation ....................................................................................................................... 27
5.1.1 Possible Explanations for Discrepancy in Carotid Flow ................................................. 67
5.2 Possible Effect of Maternal Oxygen Administration ............................................................ 69
5.3 Advantages of PC-MRI in Comparison to Other Techniques ............................................ 71
5.4 Limitations .......................................................................................................................... 73
5.5 Future Directions ............................................................................................................... 75
  5.5.1 Future Application of PC-MRI in the Sheep Fetus ........................................................ 75
  5.5.2 Maternal Hyperoxygenation as a Therapy .............................................................. 78
5.6 Conclusions ....................................................................................................................... 79
References .................................................................................................................................. 80
Copyright Statement ............................................................................................................... 99
List of Tables

Table 1. Summary of the strengths and limitations of current available techniques for measuring blood flow.

Table 2. Average fetal heart rate and blood pressure throughout MRI.

Table 3. The blood gas status of the 9 fetuses prior to induction of anesthesia for MRI scanning (top) and during the MRI scan (bottom).

Table 4. Fetal blood flows in 9 late gestation sheep fetuses acquired by PC-MRI.

Table 5. A comparison of fetal PC-MRI blood flow measurements to those previously reported in the literature.
List of Figures

Figure 1. Moving protons under an external magnetic field gradient obtains a difference in the phase of their precession (bottom), while stationary protons do not (top).

Figure 2. An example of a single frame of two-dimensional phase contrast images, obtained in a human fetus.

Figure 3. An illustration of ECG gating for cardiac MRI.

Figure 4. An example of aliasing in ascending aortic flow measurements.

Figure 5. The effect of inadequate spatial resolution on flow measurements.

Figure 6. An example of flow probes clamped around fetal cardiac vessels.

Figure 7. An example of an umbilical artery Doppler ultrasound.

Figure 8. Structure and circulation of a normal postnatal heart.

Figure 9. The fetal circulation at the level of the placenta and liver.

Figure 10. Blood flow distribution of the fetal sheep circulation.

Figure 11. Blood flow distribution of the human fetal circulation.

Figure 12. Comparison of the blood flow distribution of the fetal circulation in sheep (left) and in humans (right).

Figure 13. Examples of steady state free precession anatomical survey images in three orthogonal planes to the fetal sheep fetus.

Figure 14. An example of phase-contrast images obtained in the sheep fetus.

Figure 15. An example of typical contours used to measure the flow in fetal sheep vessels.
Figure 16. A summary of animal outcome and the number of subjects included in each stage of the study.

Figure 17. Examples of phase contrast images obtained in the sheep fetus.

Figure 18. Box plots of fetal blood flows measured by PC-MRI.

Figure 19. Blood flows in the major vessels of the sheep fetus obtained by PC-MRI.

Figure 20. Representative flow curves of targeted vessels measured by PC-MRI.

Figure 21. Intraobserver correlation between MRI flow measurements obtained by two different observers.

Figure 22. Interobserver correlation between MRI flow measurements obtained by two different observers.

Figure 23. A comparison of fetal PC-MRI blood flow measurements (left) to those previously reported in the literature (right).
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAo</td>
<td>Ascending aorta</td>
</tr>
<tr>
<td>CCA</td>
<td>Common carotid artery</td>
</tr>
<tr>
<td>CVO</td>
<td>Combined ventricular output</td>
</tr>
<tr>
<td>DA</td>
<td>Ductus arteriosus</td>
</tr>
<tr>
<td>DAo</td>
<td>Descending aorta</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DV</td>
<td>Ductus venosus</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>FO</td>
<td>Foramen ovale</td>
</tr>
<tr>
<td>ICC</td>
<td>Intra-class correlation coefficient</td>
</tr>
<tr>
<td>IVC</td>
<td>Superior vena cava</td>
</tr>
<tr>
<td>LPA</td>
<td>Left pulmonary artery</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MPA</td>
<td>Main pulmonary artery</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PBF</td>
<td>Pulmonary blood flow</td>
</tr>
<tr>
<td>PC-MRI</td>
<td>Phase-contrast cine magnetic resonance imaging</td>
</tr>
<tr>
<td>Q</td>
<td>Flow</td>
</tr>
<tr>
<td>RPA</td>
<td>Right pulmonary artery</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SVC</td>
<td>Superior vena cava</td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
</tr>
<tr>
<td>UV</td>
<td>Umbilical vein</td>
</tr>
<tr>
<td>$V_{enc}$</td>
<td>Velocity-encoding value</td>
</tr>
<tr>
<td>% of CVO</td>
<td>Percentages of combined ventricular output</td>
</tr>
</tbody>
</table>
1 Introduction

This chapter begins with an overview of the rationale for this study, explaining the potential utility of phase-contrast cine magnetic resonance imaging (PC-MRI) in the sheep fetus (Section 1.1). PC-MRI (Section 1.2), other techniques for measuring fetal blood flow (Section 1.3), and important concepts regarding the fetal circulation (Section 1.4) are then discussed.

1.1 Rationale

The fetal circulation has a unique course of distribution that is different from the postnatal circulation. Assessing the course of distribution of the fetal circulation is important for evaluating and understanding fetal physiology and helps interpret cardiovascular adaptations that might be observed in human pregnancies.

1.1.1 The Importance of Monitoring Blood Flow

Measuring blood flows in the sheep fetus has been particularly helpful for studying the fetal response to various fetal disorders, especially acute and chronic fetal hypoxemia. Fetal hypoxemia refers to reduced blood oxygen content while fetal hypoxia implies insufficient fetal oxygenation for normal fetal homeostasis, growth and development. Hypoxemia and hypoxia often occur as a result of placental insufficiency, diminished placental perfusion and umbilical cord occlusion or compression (Pearce, 2006; Richardson et al., 1998; Poudel et al., 2014). While the fetus is capable of protecting critical organ function in the setting of acute hypoxemia by increasing oxygen extraction and redistributing blood flows to supply vital organs and altering hormonal activities, chronic hypoxemia has long term effects on fetal growth and development (Pearce, 2006; Richardson et al., 1998; Poudel et al., 2014). In chronic hypoxemia, studies have shown...
that the redistribution of blood flows becomes less pronounced as the fetal requirement for oxygen becomes downregulated, with downstream growth impairment (Pearce, 2006; Richardson et al., 1998; Poudel et al., 2014). Evaluating and understanding the changes in blood flow distribution in animal models may therefore play an important role in assessing the condition of human hypoxemic fetuses.

A number of fetal sheep models of placental insufficiency have been developed. These include acute models, such as uterine and umbilical cord occlusion (Wassink et al., 2007) and ventilation of the ewe with a hypoxic gas mixture (Cohn et al., 1974) and more chronic models such as hyperthermia and uterine caruncletomy (Alexander, 1964; McMillen et al., 2001; Morrison et al., 2008). Uterine carunclectomy is a surgical procedure that removes endometrial caruncles from the uterus of non-pregnant ewes before conception. When pregnancy occurs subsequently, a reduced number of placentomes are able to form, and the sheep fetus will therefore become chronically hypoxemic throughout the gestation, due to a reduced placental oxygen and nutrient delivery (Alexander, 1964; McMillen et al., 2001; Morrison et al., 2008; Zhang et al., 2016; Poudel et al., 2014). These animal models have provided insights into circulatory adaptations to hypoxemia including the changes in vascular resistance that drive redistribution of the circulation. The increases in pulmonary vascular resistance and reductions in cerebral vascular resistance that occur in response to acute hypoxemia have been shown to correspond with changes in the Doppler waveforms in the arteries supplying the pulmonary and cerebral circulation (Makikallio et al., 2006). An understanding of the cause and effects of these changes has therefore aided the interpretation of observations made using ultrasound in human pregnancies (Wladimiroff et al., 1986). Animal models of fetal hypoxia have also been used to investigate interventions for placental insufficiency. For example, sildenafil is an antagonist to 5-hydroxytryptamine and vasodilates uterine vessels. It has been shown to protect and improve placental perfusion and increase umbilical blood flow in various animal models (Satterfield et al., 2009; von Dadelszen et al., 2011; Stanley et al., 2012; Dilworth et al., 2013; Itani et al., 2016).
1.1.2 Potential Methods for Measuring Blood Flow

Although desirable, a method for accurately and repeatedly measuring blood flow in vessels across the fetal sheep circulation is currently lacking. Perfusion of each of the major fetal organs has been successfully quantified in the sheep using radioactive microspheres while flow probes and ultrasound have been used to quantify flow in a variety of fetal blood vessels. However, each of these three techniques have their drawbacks. Flow probes involve extensive surgical procedures and are therefore not ideal for obtaining measurements under normal intrauterine condition. The radioactive microsphere technique only allows for a limited number of observations in one fetus, does not allow monitoring over several hours, and involves complex analysis in order to quantify the distribution of the whole circulation. Ultrasound measurements, although noninvasive, are often inaccurate due to the need for assumptions about the range of velocities across the vessel lumen, difficulties obtaining an adequate angle of insonation and inaccuracies in measuring vessel area (Gale et al., 2006). These techniques will be explained in more details in a later section (Section 1.3). Reliable long-term monitoring and repeated blood flow measurements over several hours to weeks across the whole fetal circulation are therefore impractical using available methods.

We therefore aimed to develop an accurate and repeatable way of measuring the distribution of blood flow across the fetal circulation in the sheep fetus under relatively normal intrauterine conditions. Our objective was to provide a method to allow the determination of changes in the distribution of flow between different fetal compartments (cerebral, pulmonary, systemic and umbilical) for use in acute and chronic models of placental insufficiency. We identified PC-MRI as a potentially feasible approach for this goal as PC-MRI is a noninvasive tool that allows for the accurate quantification of vessel blood flow by exploiting the change in magnetization experienced by protons moving along a magnetic gradient. In the past decade, PC-MRI has become the clinical gold-standard non-invasive technique for measuring vessel blood flow. PC-MRI is often applied to evaluate cardiovascular disorders including the quantification of valvular regurgitation, shunts and vessel stenosis (Lotz et al., 2002). Although PC-MRI has been performed
successfully in human fetuses, limited investigation of its feasibility in fetal sheep have been undertaken. Schoennagel et al. (2013) performed a pilot study attempting PC-MRI in the descending aorta of 5 sheep fetuses using an ultrasound trigger. However, in this study no attempt was made to characterize the distribution of the fetal circulation. To the best of our knowledge, there has been no other study in which PC-MRI was applied the sheep fetus.

1.2 PC-MRI

To understand the nature of this study, a basic understanding of PC-MRI is important. This section will therefore explore the current state of knowledge regarding PC-MRI.

1.2.1 Basics of PC-MRI

MRI involves the application of radiofrequency energy in a strong magnetic field that results in tipping of the magnetic moment of protons in the subject away from the longitudinal axis of the magnetic field (McRobbie et al., 2006). As the magnetic moment rotates into a transverse plane, it induces an electric current in the coils positioned around the subject. A series of magnetic field gradients allows this MRI signal to be spatially encoded, and Fourier transforms convert the MRI signal into an image. MRI acquisitions are accomplished using a pulse sequence, stored in the scanner computer, which determines what qualities of the tissues are interrogated.
Figure 1. Moving protons under an external magnetic field gradient obtains a difference in the phase of their precession (bottom), while stationary protons do not (top). The phase difference is proportional to the velocity of the protons (Reproduced from Lotz et al., 2002).
PC-MRI allows measurement of blood flow because it creates contrast between stationary tissue surroundings and flowing blood by manipulating the phase of the transverse magnetization in order to acquire contrast between stationary tissue surroundings and flowing blood (Korosec, 1999). The phase is determined by how far the magnetization precesses from the time it is oriented into the transverse direction until the time it is detected (Korosec, 1999). The phase of magnetization is manipulated in such a way that the phase from the moving protons is non-zero, whereas that from the stationary protons is zero (Figure 1, Lotz, 2002). After processing, phase-difference images can be produced as a result. In phase-difference images, the signal obtained is proportional to the amount of phase difference, which in turn is proportional to the velocity of the moving protons (Korosec, 1999). The faster the protons move, the larger the signal is. And protons moving in a certain direction are arbitrarily assigned a bright signal; protons moving in the opposite direction are given a dark signal; and stationary tissues appear grey in the images (Korosec, 1999). From phase-contrast images, the vascular anatomy can be observed and evaluated, while quantitative assessment of the blood’s volume flow rate and velocity can also be accomplished (Figure 2). Figure 2 shows an example of a single frame of phase-contrast images of a blood vessel.

In order to achieve accurate vessel blood flow measurements with PC-MRI, certain criteria should be met. Where possible, turbulent flow should be avoided as this degrades the accuracy of the measurements. Importantly, the scan parameters should afford adequate spatial and temporal resolution. Sufficient temporal resolution allows discrimination between the different flow velocities present during different phases of the cardiac cycle, while adequate spatial resolution will account for variation in flow velocity across the vessel lumen. For example, blood flow is typically faster in the centre of the vessel and thus adequate spatial and temporal resolution are required to distinguish the differences. Typical scan parameters for high quality PC-MRI flow measurements result in at least 8 pixels over the vessel lumen and a temporal resolution of at least 50 ms. To quantify vessel flow, the two-dimensional PC-MRI imaging plane needs to be prescribed as either orthogonal or perpendicular to the direction of flow.
PC-MRI is sensitive to a range of blood flow velocities and therefore requires specification of this range to acquire accurate measurements (Korosec, 1999). The operator must choose an appropriate velocity-encoding value ($V_{enc}$). A $V_{enc}$ that is close to the maximum expected velocity, also known as the ideal $V_{enc}$, results in blood flow measurements with good accuracy (Korosec, 1999). Thus, a different $V_{enc}$ should be used to image different vessels in different scans.

After the scan, the phase difference between stationary tissue and flowing blood of the vessel should be processed in order to obtain and quantify flow throughout the cardiac cycle. The data acquired from phase-contrast measurement is converted into two sets of images: a magnitude image used for anatomic orientation, and a velocity image in which the gray value of each pixel encodes the flow value (Lotz et al., 2002). Commercial software programs can then allow for the quantitative analysis of the sets of through-plane PC-MRI images to obtain blood flow measurements. Two examples of such software programs are QFlow (Medis, Leiden, the Netherlands) and CVI42 (Calgary, Canada).
Figure 2. An example of a single frame of two-dimensional phase contrast images, obtained in a human fetus. The image is prescribed perpendicular to the ascending aorta in order to obtain its flow. The data are displayed as a pair of images with the flow information on the left (velocity image) and the corresponding anatomy on the right (magnitude image). The fetal ascending aorta is circled in red.

1.2.2 Fetal MRI and Cardiac Gating

Although PC-MRI is a well-established technique for quantifying blood flow in the postnatal circulation, its use in the fetus remains rather limited. Fetal MRI can be performed safely at 1.5 Tesla (T) and 3.0 T (Seed, 2015). 1.5 T MRI, which was eventually employed in this study, is less prone to banding artifacts that result from field inhomogeneity (Seed, 2015).

In order to achieve adequate spatial and temporal resolution to make accurate flow measurements, cardiac triggering or “gating” is required. This is because there is not sufficient time to collect the entire high resolution image at each cardiac phase of a single cardiac cycle. Gating allows an image to be created for each cardiac phase by combining
the data acquired over many cardiac cycles. There are two types of cardiac gating: prospective and retrospective. Prospective gating relies on a trigger signal to start acquiring data (Lotz et al., 2002). When each cardiac cycle ends, data acquisition is temporarily paused until the next trigger signal. This waiting interval is known as the arrhythmia rejection window, and is employed to compensate for any variation in the length of each cardiac cycle (physiological arrhythmia) during the scan (Lotz et al., 2002). However, some of the late diastole flow may not be captured entirely as the acquisition over any cardiac cycle is truncated to a constant length (Lotz et al., 2002).

With retrospective gating, data are acquired continuously throughout the entire cardiac cycle (Lotz et al., 2002). The trigger signals are still recorded, but are only used to organize the data retrospectively by assigning them to the different corresponding positions in the cardiac cycle during image construction (Lotz et al., 2002). Because physiologic variations in the length of the cardiac cycle are always present, data interpolation is needed to represent average flow over a cardiac cycle (Lotz et al., 2002). This leads to small inaccuracies. However, a major advantage of retrospective gating is that the data acquisition can cover the entire cardiac cycle (Lotz et al., 2002). Whether prospective or retrospective gating is applied depends on the sequence and the MR imaging systems (Lotz et al., 2002). In order to capture flow throughout the cardiac cycle, a retrospective gating technique was employed for the fetal sheep imaging in the current study.

For both prospective and retrospective gating, a gating trigger is required for image reconstruction. In postnatal subjects, traditional gating triggers include electrocardiogram (ECG) and pulses (Tseng et al., 2016). In ECG triggering, a specific point of the ECG, usually the R wave, is used as a trigger signal, such that data acquisition can be initiated following a given delay after the R wave in prospective gating (Tseng et al., 2016; Figure 3). Figure 3 shows an illustration of ECG gating. In pulse triggering, the pulse wave can be used as a trigger signal in place of the R wave in electrocardiogram (Tseng et al., 2016). However, placement of the thoracic surface electrodes for ECG gating or finger probe for pulse gating is not feasible for fetal sheep imaging because the fetus is within the uterus.
and cannot be accessed. Alternative approaches are therefore required for triggered cardiac imaging in the fetus.

Several groups have proposed different techniques for gating in the fetus that can be broadly categorized into non-invasive and invasive techniques. Non-invasive techniques can be used for human and animal studies, and include metric optimized gating, ultrasound-based gating and self-gated approaches. Metric optimized gating, a retrospective gating technique developed by Jansz et al. (2010) reconstructs data acquired with an artificial trigger through a range of candidate heart rates, identifying the most appropriate reconstruction through the lack of artifact in the final images. Ultrasound based gating techniques using cardiotocographic monitoring modified for use in an MRI environment have been described in animal models (Feinberg et al., 2010; Yamamura et al., 2015; Kording et al., 2014; Schoennagel et al., 2014). “Self-gating” detects motion based on periodic variations within the MR data itself (Nieman et al. 2009; Yamamura et al., 2010).

Invasive techniques have been described employing blood pressure variation across the cardiac cycle to trigger cardiovascular imaging, with surgical placement of an arterial catheter prior to imaging have been described (Yamamura et al., 2009). The blood pressure of the fetus is transduced from the arterial catheter and relayed to the MRI scan computer for use as a pulse trigger. Unlike the non-invasive techniques described above, invasive gating offers a reliable triggering signal with no requirement for additional equipment or post-processing techniques. Arterial cannulation is a well-established technique in fetal sheep, and can be maintained for extended periods when the arterial lines are exteriorized via a small incision in the maternal abdominal wall. Given the large amount of data we planned to collect in order to determine the distribution of the fetal circulation, and our prior experience with fetal sheep surgery, we opted for invasive blood pressure transduction as our means of obtaining triggering cardiac imaging for the current experiment.
Figure 3. An illustration of ECG gating for cardiac MRI. Images were obtained at different cardiac phases throughout the cardiac cycle. The image at each cardiac phase is reconstructed from pieces of data acquired over multiple heartbeats.

1.2.3 Accuracy of PC-MRI Flow Measurements

Many groups have investigated the accuracy of PC-MRI flow measurements in adults. Evans et al. (1993) determined a difference of 5% between ascending aorta and pulmonary artery flow in healthy adults. They estimated a 3.5% to 4.5% deviation from the true flow as a result of technical error, using a sequence with no breath holding and prospective gating. Kondo et al. (1991) determined a similar difference between aorta and pulmonary artery flow in adults. Lotz et al. (2002) measured differences of up to 3% between aorta and pulmonary artery flow in adults, using a sequence with retrospective gating and breath holding with an intraobserver variability of 2% and interobserver
variability of 3%. These data would suggest that under optimal conditions PC-MRI is a highly reliable technique for measuring blood flow in the great vessels of adults.

In human fetuses, Seed et al. (2012) determined good reproducibility ($R = 0.96, P < 0.001$) and interobserver agreement ($R = 0.99, P < 0.001$; bias = $2.38 \pm 34.86$ ml/min) with PC-MRI flow measurements using metric optimized gating. Good agreement ($R = 0.90, P = 0.002$) was found between indirect (main pulmonary artery – ductus arteriosus) and direct measurements of pulmonary blood flow (right + left pulmonary artery flow). Prsa et al. (2014) also found good reproducibility ($R = 0.96, P \leq 0.0001$, bias = $10.8 \pm 71.3$ ml/min) and interobserver agreement ($R = 0.97, P = 0.0001$, bias = $-21.2 \pm 48.3$ ml/min) using metric optimized gating in a larger group of fetuses. The direct and indirect measurements of pulmonary blood flow showed good agreement with no significant bias ($R = 0.43$, $P = 0.004$, bias = $10.5 \pm 56.0$ ml/min/kg). These findings suggest that PC-MRI flow quantification in the large vessels of late gestation human fetuses is feasible.

1.2.4 Potential Sources of Errors in PC-MRI Measurements

One potential source of error in PC-MRI flow measurements is encountered when a $V_{\text{enc}}$ value below the ideal $V_{\text{enc}}$ results in aliasing (Figure 4; Srichai et al., 2009). However, a $V_{\text{enc}}$ value above the ideal $V_{\text{enc}}$ would result in lower signal and therefore less accuracy, and the range of flow would become more narrow as a result (Srichai et al., 2009). Errors in the selection of the correct $V_{\text{enc}}$ can be overcome when the normal range of flow velocities are known, or by repeating the imaging with a new $V_{\text{enc}}$ when aliasing occurs or the $V_{\text{enc}}$ is too high.

Deviations from an ideal imaging plane can also lead to errors in PC-MRI flow measurement. PC-MRI flow measurements are the most precise when the prescribed imaging plane is orthogonal to the direction of flow and the flow encoding is through-plane (Lotz et al., 2002). An oblique plane prescription is usually noticeable from the anatomy shown in the magnitude image or shape of the vessel, which is most likely to be...
circular when orthogonal. Deviation from an orthogonal imaging plane results in excessive partial volume effects, impeding the accuracy of flow measurements. However, deviation of less than 15 degrees from the orthogonal plane is generally acceptable (Tang et al., 1993).

Inadequate spatial resolution can also affect the accuracy of PC-MRI flow measurements because partial volume effects lead to the underestimation of flow (Figure 5). If the pixel size is more than 1/3 of the vessel diameter, the accuracy of PC-MRI flow measurements are subject to partial volume effects (Lotz et al., 2002). A fetal vessel is expected to be around 5-10 mm in diameter in late gestation. For a vessel diameter of 5 mm, 1.67 mm in-plane resolution is therefore required. With a typical field of view for fetal PC-MRI of around 240 × 320 mm, a matrix size of around 144 × 192 mm is required.

Other common potential sources of errors include field inhomogeneity (eg. Artifacts from surrounding tissues), turbulent flow in the vessel, pulsation artifacts, and spatial misregistration (eg. Fetal movement) (Srichai et al., 2009; Lotz et al., 2002).
Figure 4. An example of aliasing in ascending aortic flow measurements. Shown here are magnitude images (top), velocity images (middle), and blood velocity profile plots (bottom) of measurements with (left, arrow, $V_{\text{enc}} = 100$ cm/s) and without (right, $V_{\text{enc}} = 120$) aliasing (Reproduced from Lotz et al., 2002).
Figure 5. The effect of inadequate spatial resolution on flow measurements. Data plotted were obtained from an experiment with constant laminar flow of -2200 ml/min. The spatial resolution was gradually decreased. Green area shows a deviation of ± 10% from the flow at the highest spatial resolution in order to demonstrate the acceptable range. FOV, field of view. (Reproduced from Lotz et al., 2002).

1.3 Other Methods of Measuring Blood Flow

PC-MRI is a relatively new method for measuring the flow in blood vessels. In 1967, Rudolph and Heymann (1967) developed a technique to measure fetal blood flow under normal physiological conditions, using radionuclide-labeled microspheres. Fetal blood flow has also been measured using electromagnetic and ultrasonic flow probes. Doppler ultrasound has also been employed as a non-invasive alternative for measuring fetal vessel blood flows. Each of these methods have their unique values and limitations, which are discussed in more details below.
1.3.1 Flow Probes

Flow probes are small devices that are placed around a target vessel to interrogate the flow within the vessel. Two types of flow probes have been used in the fetus: electromagnetic flow probes and ultrasonic flow probes. The electromagnetic flow meter technique was employed in the exteriorized fetus by Berman et al. (1975). It works through generating a magnetic field across a vessel and measuring the voltage generated as blood flow disturbs the magnetic field. This method was first used in the exteriorized fetus but were later employed as a chronic instrument clamped around the vessel of interest to monitor blood flow. Ultrasonic flow probes transmit sonic waves between pairs of sensors and thereby allow the estimation of the blood flow velocity from the differential transit time from one sensor to the other. To measure flow, the fetus needs to undergo surgery to access the vessel and place the flow probe around it (Figure 6). The blood flow velocity and the estimated or known diameter of the vessel are used to calculate blood flow.

The flow probe technique is often employed to measure blood flow in one or two fetal vessels for each fetus, commonly the fetal carotid artery, femoral artery, umbilical artery and pulmonary artery. Nimrod et al. (1989) studied blood flow in fetal umbilical and carotid arteries using electromagnetic flow probes. Clark et al. (1992) studied blood flow in the umbilical artery in response to maternal nicotine administration using electromagnetic flow probes. Wassink et al. (2007) studied flow in the femoral artery using ultrasonic flow probes with prolonged umbilical occlusion. Bennet et al. (2007) studied blood flow using ultrasonic flow probe in the femoral artery and carotid artery in response to umbilical occlusion. Smolich et al. (1985) studied ventricular output in response to early cord clamping. Other groups have studied the effect of various factors, such as oxygen tension and endothelin on pulmonary artery blood flow using ultrasonic flow probe (Tiktinsky et al., 1993; Wong et al., 1994). Electromagnetic flow probes are also commonly employed for measuring the flow and resistance index of the umbilical artery (Stek et al., 1993; Lang et al., 2000).
Figure 6. An example of flow probes clamped around fetal cardiac vessels. Ao F, ascending aortic fluid-filled catheter; AI FP, aortic isthmus flow probe; Ao M, ascending aortic micromanometer catheter; Duct FP, ductus arteriosus flow probe; LPA FP, left pulmonary artery flow probe; LV, left ventricle; PT M, pulmonary trunk micromanometer catheter; PT F, pulmonary trunk fluid-filled catheter; DTA, descending thoracic aorta; RV, right ventricle (Reproduced from Smolich et al., 1985).

The flow probe technique has commonly been used for determining flow in response to various interventions in the sheep fetus. It is a valuable technique for measuring blood flow, being particularly useful for monitoring flow in certain target vessels over several hours or even weeks. Chronic instrumentation also allows for the measurement of blood flow under relatively normal intrauterine condition. However, one major drawback of the flow probe technique is its requirement for installation of a probe on every target vessel. This makes measuring flow in multiple vessels at the same time particularly difficult, due to the complexity and invasiveness of the surgical procedures.
required to access the fetal vessels. Flow probes are therefore unsuitable for assessing the fetal circulatory distribution in a comprehensive manner. Also, the fetal vessel may outgrow the flow probes and will require replacement or adjustment for monitoring blood flows over weeks. The instrumentation of the flow probes may affect the fetal condition, confounding any research findings. Furthermore, it is not possible to allow spontaneous delivery of the fetus with flow probes in place.

1.3.2 Labeled Microspheres

Another technique often used to measure blood flow distribution in the sheep fetus is known as the radionuclide-labeled (or radioactive) microsphere technique. The radionuclide-labeled microsphere technique was developed by Rudolph and Heymann in 1967 for measuring organ blood flow distribution in the fetus. The original microsphere technique first requires the insertion of catheters into fetal umbilical, forelimb and hindlimb veins through a fetal catheterization surgery (Rudolph & Heymann, 1967). The fetus is then allowed time to recover from the surgery. Then, in the standing and unanesthetized ewe, antipyrine is infused into a fetal limb vein in order to measure the placental-arteriovenous difference of antipyrine (Rudolph & Heymann, 1967). The difference measured can then be used to calculate the fetal umbilical blood flow by the Fick method by obtaining a blood sample from the umbilical fetal venous catheter (Rudolph & Heymann, 1967). The Fick principle states that the uptake or release of a substance by the placenta is the product of the arteriovenous concentration difference of the substance and the blood flow to that organ.

After the blood sample is taken, radionuclide-labeled microspheres that are roughly 50 µm in diameter are injected into forelimb, umbilical and hindlimb veins through catheters (Rudolph & Heymann, 1967). The microspheres will then be trapped in the capillaries of the end organs. The nuclides that are commonly used predominantly have gamma emissions, usually a combination of two or more of iodine-125, chromium-51,
strontium-85, cerium-141, niobium-95 and scandium-46 (Rudolph & Heymann, 1970; Rudolph & Heymann, 1967; Rudolph et al., 1971). The sheep are then humanely killed to measure the distribution of the microspheres in the organs by desiccating the entire organs and detecting their nuclide count (Rudolph & Heymann, 1967). The nuclide counts in each organ combined with the umbilical blood flow measurements allow the calculation of blood flow in organs of the lower body. Blood flow through the inferior vena cava, superior vena cava and upper body organs can then be deducted in series from the distribution of the microspheres and blood flows to the lower body organs. Rudolph and Heymann applied this technique to measure cardiac output and fetal blood flow to various organs and vessels, including the inferior vena cava, superior vena cava, placenta, brain, kidneys, gut, spleen, heart, liver, lungs, lower carcass and upper carcass in the normal sheep at various gestational ages (Rudolph & Heymann, 1970; Rudolph & Heymann, 1967).

Since the initial proposal of this radionuclide-labeled microsphere technique, several modifications have been presented. One modified technique was known as the reference sample method (Heymann et al., 1977; Schmidt et al., 1991). Instead of quantifying the umbilical blood flow using antipyrine infusion and the Fick principle, reference blood samples are obtained before, during and after the injection of 15-µm diameter plastic microspheres from reference fetal vessels, from both upper and lower body (e.g. Carotid arterial and femoral arterial catheters), to measure fetal blood flow distribution. The radioactivity of the reference blood samples and organs are measured. Blood flow can be calculated using the following formula, \( Q_{\text{organ}} = \frac{(Q_{\text{reference}} \times \text{organ counts})}{\text{reference counts}} \), where \( Q \) is blood flow.

In place of radioactive microspheres, fluorescent microspheres can be injected using a very similar approach into the fetus (Poudel et al., 2015). Instead of radioactivity, the fluorescent intensity of the samples is measured to determine blood flow. This technique has demonstrated high sensitivity and good spectral separation and allows a relatively time-saving microsphere isolation process (Prinzen & Glenny, 1994).
The absence of recirculation of the injected radionuclide-labeled microspheres demonstrated the accuracy of the microsphere technique (Rudolph & Heymann, 1967). The distribution of microspheres has also been shown to be proportional to blood flow (Rudolph & Heymann, 1967). Importantly, the circulatory physiology of the sheep fetus appeared to be unaltered by the injection of microspheres (Rudolph & Heymann, 1967).

The microsphere technique thus has a unique advantage of allowing the reliable and comprehensive measurement of fetal blood distribution under normal physiological conditions, with no administration of anesthesia or oxygen to the ewe needed. The blood flow determined by this technique has therefore been treated as the gold standard measurement for normal sheep fetuses. However, the microsphere technique does not allow for monitoring of fetal blood flow status over a period of several hours. Its ability to measure blood flow repeatedly is also limited by the availability of different types of radioactive nuclides that can be injected into a single animal and separated in a single sample. The radioactivity of the microspheres may also decay over time, with most of the commonly used isotopes having a half life of between ~30 and ~90 days, affecting its reliability of monitoring blood flow over several weeks. The application of the microsphere technique to the repeated measurements of blood flow over a prolonged period, and the examination of the effects of interventions are therefore somewhat limited.

1.3.3 Doppler Ultrasound

Another way to measure fetal vessel blood flow is to use Doppler ultrasound. Ultrasound imaging is based on the generation of sound waves which are reflected by the body’s tissues. Doppler ultrasonography is based on the Doppler effect whereby the incident frequency of the sound wave passing through the subject is different from the frequency of the reflected echo (Evans et al., 1989). The Doppler ultrasound transducer sends a series of pulses into the target area to detect the movement of fluid. While stationary tissues give the same echo from pulse to pulse, echoes from moving blood have
differences in the time it takes for the echo to be returned to the receiver (Evans et al., 1989; Atkinson & Wells, 1977). The differences can be detected as direct time differences or a phase shift to the Doppler frequency (Evans et al., 1989). These can then be processed to give blood velocity. The higher the blood velocity, the higher the Doppler frequency (Evans et al., 1989). The quality of the ultrasound images relies on the choice of ultrasound frequency used. The higher the ultrasound frequencies, the higher the sensitivity to flow; the lower the ultrasound frequencies, the better the penetration (Evans et al., 1989). As a result, when choosing the correct ultrasound frequency, a balance between sensitivity and penetration needs to be achieved for optimal image quality. Blood flow can be calculated from the product of fetal heart rate, the time velocity integral of the traces detected by Doppler ultrasound and the cross-sectional area of the vessel (calculated from vessel diameter measured by ultrasound).

Doppler ultrasound is performed in the human fetus as a clinical routine to noninvasively evaluate placental perfusion abnormalities and fetal hemodynamics (Figure 7). It is a valuable tool for evaluating fetal hemodynamics in congenital heart diseases and intrauterine growth restrictions. Commonly examined fetal vessels beside the fetal heart include the ductus venosus (Cruz-Martines & Figueras, 2009), the umbilical arteries (Fisk et al., 1988), and the middle cerebral artery (Wladimiroff et al., 1986; Hershkovitz et al., 2000; Severi et al., 2002) to assess intrauterine growth restriction. In the sheep fetus, Schmidt et al. (1991) determined fetal ascending aorta and umbilical blood flow using Doppler ultrasound. Doppler has also been applied to monitor blood flow velocity in the umbilical vein, ductus venosus, umbilical arteries, and common carotid arteries in response to hypoxemia during early gestation (Kiserud et al., 2001). It has been used to obtain the blood velocity waveforms of umbilical artery, descending aorta, ductus arteriosus, aortic isthmus, and proximal right or left pulmonary artery in late-gestation chronically hypoxic sheep fetuses (Makikallio et al., 2006).

The major advantage of Doppler ultrasound for measuring fetal blood flow lies in its noninvasive nature, which has resulted in its widespread application in clinical obstetrics. However, the accuracy of ultrasound is limited by its reliance on the estimation
of the cross-sectional area of a target fetal vessel and assumption of a constant velocity over the entire vessel area, which often results in overestimation of flow (Lotz et al., 2002; Schimdt et al., 1991). In adult blood vessels, it has been shown that PC-MRI flow measurements are more accurate than Doppler ultrasound measurements in normal volunteers (Lotz et al., 2002).

In summary, each of the above discussed techniques for measuring blood flow are invaluable for different types of research (Table 1). While flow probes can measure flow repeatedly over several hours, days or weeks, thus facilitating assessment of the effects of interventions, they rely on complex surgical procedures and commercialized flow probe products for every fetal vessel. They are therefore unsuitable for comprehensive assessment of the fetal blood flow distribution. Radionuclide-labeled microspheres allow the comprehensive assessment of the fetal circulation under normal intrauterine condition, but they are somewhat unsuitable for monitoring the effect of interventions and repeated measurements over a prolonged period. While Doppler ultrasound is not invasive and allows for the comprehensive assessment of the fetal circulation, it is more prone to inaccuracy compared to PC-MRI. Taking CVO measurements as an example, De Smedt (1987) determined a CVO of $553 \pm 153$ ml/min/kg in 28 normal human fetuses near term using two dimensional Doppler echocardiography. Mielke et al. (2001) found a CVO of $429 \pm 100$ ml/min/kg in 37 normal human fetuses at 23-40 weeks gestation. Kiserud et al. (2006) determined a CVO of around 400 ml/min/kg in 170 fetuses at 18-40 weeks gestation. We therefore hypothesized that PC-MRI could provide a promising alternative to these established methods for achieving a comprehensive assessment of fetal circulatory distribution and be useful for long-term monitoring and the assessment of the effect of interventions. Although our approach does involve fetal surgery for placement of an arterial catheter, the fetus can be allowed a period to recover following surgical intervention. However, procedural sedation and maternal oxygen ventilation are required for fetal MRI in the sheep, which could have an impact on fetal circulatory hemodynamics.
Figure 7. An example of an umbilical artery Doppler ultrasound. The image was obtained in a normal human fetus at 38 weeks’ gestation.
Table 1. Summary of the strengths and limitations of current available techniques for measuring blood flow.

<table>
<thead>
<tr>
<th></th>
<th>Invasiveness</th>
<th>Normal Intrauterine Condition</th>
<th>Detecting Acute Changes</th>
<th>Detecting Chronic Changes</th>
<th>Reliability</th>
<th>Comprehensive Distribution Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Probes</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Radionuclide-labeled Microspheres</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Doppler Ultrasound</td>
<td>–</td>
<td>+ (Anesthesia and ventilation required)</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PC-MRI</td>
<td>+ or − (for gating)</td>
<td>+ (Anesthesia and ventilation required)</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>
1.4 Fetal Circulation

This section will explore the current state of knowledge regarding the late gestation prenatal circulation under normal intrauterine conditions. The main purpose of the circulation is to serve as a transportation system across the body in order to provide nutrients and oxygen to all cells, remove metabolic waste, and support growth and development. Because of its unique intrauterine environment, the fetal circulation is similar to and yet distinct from the postnatal circulation. Understanding the normal fetal circulation is important for defining and understanding the causes and pathologies behind many pregnancy disorders with affected fetal circulation, such as congenital heart diseases and intrauterine growth restriction. In order to understand the fetal circulation and its unique adaptations to the prenatal environment, a basic understanding of the postnatal circulation is required.

1.4.1 Postnatal Circulation

Postnatally, the venous blood returns to the right atrium through the superior vena cava (SVC) and the inferior vena cava (IVC), respectively from the upper and lower body. The venous blood then passes through the tricuspid valve, enters the right ventricle and then drains into the main pulmonary artery (MPA). The MPA then separates into left and right branch pulmonary arteries (LPA and RPA), which carry the blood to the lungs. In the normal postnatal condition, the mature lungs allow for gas exchange, and the newly oxygenated blood returning from the lungs then enters the pulmonary veins. The oxygenated blood enters the heart through the left atrium and is pumped into the systemic circulation by the left ventricle. The blood from the left ventricle travels through the ascending aorta (AAo), which, after branching into brachiocephalic artery, left common carotid artery (CCA) and left subclavian artery, drains into the descending aorta (DAo). The DAo runs down through the chest and abdomen, giving rise to branch arteries that supply oxygenated blood to the lower half of the body. As it passes through the various
organs, the blood becomes deoxygenated and returns to the heart through the systemic venous system. Aside from a small amount of coronary venous blood that enters into the left ventricle and bronchial venous blood that enters into the pulmonary veins, there is no mixing of oxygenated and deoxygenated blood in the postnatal circulation. Nutrients, in the form of metabolic substrates, are absorbed from the gastrointestinal tract and delivered to the liver for processing through the portal venous system. Figure 8 shows an illustration of the normal postnatal circulation at the level of the heart.

Figure 8. Structure and circulation of a normal postnatal heart. Blue arrows represent the direction of flow of de-oxygenated blood. Red arrows represent the direction of flow of oxygenated blood.
1.4.2 Fetal Circulation

The hemodynamics and course of blood distribution in the sheep fetus were established by Rudolph et al. (2009) using radionuclide-labeled microsphere studies. The fetal circulation is distinct from the postnatal circulation in part because gas exchange occurs at the placenta, rather than at the lungs (Rudolph, 2009). The deoxygenated fetal blood is delivered to the placenta for oxygenation through the umbilical arteries. The oxygenated blood then returns to the fetus through the umbilical vein (UV). The UV enters the body at the hilum of the liver, through the umbilicus (Rudolph, 2009). The UV then gives rise to branches that perfuse the left lobe of the liver, and divides into the ductus venosus (DV) and an arcuate branch that supplies blood to the right lobe of the liver (Figure 9; Lind et al., 1964; Rudolph, 2009). The DV is unique to the fetal circulation for oxygenated blood from the UV to enter the circulation (Rudolph, 2009). The DV runs towards the dorsal side of the fetal body, joining the inferior vena cava (IVC). The left hepatic vein enters into the IVC at the same junction, joining the flow from the DV. The right hepatic vein also enters the IVC but separately at a different junction site (Rudolph, 2009). Both these junctions, known as orifices, are covered by a valve-like membrane caudally. Although their functions are not well-understood yet, these valve-like membranes may play an important role in facilitating the unidirectional flow of the hepatic venous and UV streams entering the IVC (Rudolph, 2009).

Blood from both the IVC and the SVC, which collect venous blood from the upper body including the brain, enters the right atrium just as in postnatal circulation (Rudolph, 2009). However, after entering into the IVC, the oxygenated blood from the DV is preferentially streamed across the foramen ovale (FO), an opening between the left and right atrium unique to the fetus (Rudolph, 2009). This preferential streaming allows the well-oxygenated blood to bypass the right atrium and ventricle and enter directly into the left atrium (Rudolph, 2009). Only a small proportion of the blood from DV is not preferentially streamed across the FO and goes into the right heart, entering the right ventricle. In contrast, blood from the abdominal IVC is preferentially directed to travel through the tricuspid valve and enters the right ventricle (Rudolph, 2009). And only a
small proportion of the blood from abdominal IVC is preferentially streamed across the FO. On the other hand, the majority of the venous blood from the SVC mainly passes through the tricuspid valve and enters the right ventricle, while a small proportion of about 5% passes across the FO (Rudolph, 2009).

**Figure 9. The fetal circulation at the level of the placenta and liver.** The umbilical vein branches to perfuse the left lobe of the liver, and then divides into the ductus venosus and an arcuate branch that supplies blood to the right lobe of the liver. The ductus venosus, left hepatic vein and right hepatic vein join the inferior vena cava. IVC, inferior vena cava; LHV, left hepatic vein; RHV, right hepatic vein; SVC, superior vena cava (Reproduced from Rudolph, 2009).
The blood that passes through the tricuspid valve into the right ventricle is pumped into the MPA (Rudolph, 2009). However, unlike in the postnatal circulation, the blood from the MPA does not simply drain into RPA and LPA and enter the lungs. Instead, another shunt, known as the ductus arteriosus (DA), shunts the blood from the MPA into the DAo, joining the blood from the left ventricle and the AAo (Rudolph, 2009). A small proportion of blood from the MPA goes into the branch pulmonary arteries, while the majority of blood passes through DA and enters the systemic circulation directly. The blood that enters the branch pulmonary arteries returns to the left atrium through the pulmonary veins. It is then pumped out of the left ventricle through the AAo (Rudolph, 2009). The majority of the AAo blood passes into branches to supply the upper body while a small proportion passes into the DAo, joining the blood shunted through the DA. The majority of the DAo blood enters into the umbilical-placental circulation for gas and nutrient exchange; and a small proportion supplies blood to the lower body, including the abdominal organs and lower limbs (Rudolph, 2009). Due to the differences in vessel connections and gas exchange site, the fetal circulation has different cardiac output and course of distribution from the postnatal circulation.

1.4.3 Fetal Cardiac Output and Distribution

In the postnatal circulation, cardiac output is defined as the volume of blood going through the heart per unit time and has traditionally been used as an important measurement of heart function. In the fetus, the right and left ventricular outputs are different from the postnatal circulation because they are in parallel rather than in series. To facilitate better understanding of the fetal blood flow distribution, it is therefore customary to express blood flow as percentages of the combined ventricular output (CVO), the sum of output volumes of the two ventricles.
The blood flow in major vessels, expressed as percentages of CVO (% of CVO), have been well-established in both human and sheep fetuses. Rudolph et al. (1970) determined the blood flows of chronically instrumented sheep fetuses using radionuclide-labeled microspheres. To summarize, CVO varies with gestational age, reaching about 400-550 ml/min/kg fetal weight at late gestation. The blood flows in the major vessels determined by this technique are presented in Figure 10. Around 90 ml/min/kg of blood returns from the SVC while ~310 ml/min/kg returns from the IVC. Approximately 115 ml/min/kg of the IVC blood is streamed across the FO, while the remaining ~195 ml/min/kg from IVC joins the SVC flow and passes through the right ventricle at a rate of ~300 ml/min/kg into the MPA. 265 ml/min/kg of the MPA flow is shunted through the DA into the descending aorta, while 35 ml/min/kg traverses the pulmonary circulation and returns to the left atrium. The pulmonary blood flow joins the FO flow in the left atrium and passes into the left ventricle resulting in ~150 ml/min/kg being pumped out by the left ventricle, with 15 ml/min/kg going to the coronary circulation and the rest to the head and neck vessels and the DAo.

In human fetuses, blood flow distribution has been studied using ultrasound and PC-MRI. The measurements obtained by ultrasound from numerous studies are somewhat variable (De Smedt et al. 1987; Sutton et al., 1994; Chaoui et al., 1995; Rasanen et al., 1996; Mielke et al., 2001; Rasanen et al., 1998; Kiserud et al., 2006). PC-MRI flow measurements have been shown to be more reliable in adult vessels than ultrasound. Lotz et al. (2002) found that the PC-MRI flows in the AAo and MPA agreed to < 3% in normal adults. The blood flows in major fetal vessels have been determined by Prsa et al. (2014) using PC-MRI in 40 normal human fetuses at late gestation (Figure 11). The study found a mean CVO of 465 ± 57 ml/min/kg. The course of distribution of human fetal circulation measured by PC-MRI is presented in Figure 11 and described as follows.
Figure 10. Blood flow distribution of the fetal sheep circulation. The blood flows were expressed as mean flows on the right and as mean percentages of the combined ventricular output on the left (Reproduced from Rudolph, 2009).

Around 138 ml/min/kg of blood returns from the SVC while ~250 ml/min/kg returns from the IVC (Prsa et al., 2014). ~133 ml/min/kg of the IVC blood is streamed through the FO, while the remaining joins the SVC flow and passes through the right ventricle at a rate of ~264 ml/min/kg into the MPA (Prsa et al., 2014). ~186 ml/min/kg of the MPA flow is shunted through the DA into the DAo, while ~77 ml/min/kg traverses through the pulmonary circulation and returns to the left atrium (Prsa et al., 2014). The pulmonary blood flow joins the FO flow in the left atrium and passes into the left ventricle.
Approximately 192 ml/min/kg is pumped out by the left ventricle, with ~13 ml/min/kg estimated for coronary blood flow based on Rudolph’s sheep observations. The remainder passes into the head and neck vessels and the DAo (Prsa et al., 2014).

There are marked differences between sheep and human fetal circulations in terms of left and right ventricular output (Figure 12). While both species demonstrate more output from the right ventricle than the left, the difference is much more pronounced in the sheep fetus. Partly as a result of the much higher right ventricular output, flow across the DA is also much larger in the sheep than the human fetus (59 in the sheep versus 41 % of CVO in the human) (Rudolph, 2009; Prsa et al., 2014). Another factor contributing to the larger DA flow found in the sheep is the lower PBF (7 versus 16 % of CVO). Also, the sheep circulation demonstrates more blood flow going to the lower systemic circulation through the DAo (52 versus 59 % of CVO) and less to the brain and returning through the SVC (21 versus 29 % of CVO) (Rudolph, 2009; Prsa et al., 2014). These differences in blood flow distribution between the two species can largely be attributed to the relative size of the important organs, particularly brain size. In the late gestation fetus, the brain constitutes around 12% of fetal body weight in humans versus only 3% in the fetal sheep at late gestation (Rudolph, 2009). The organ blood flow after indexing to the organ weight is therefore likely similar in the two species. Sheep fetuses, which have similar course of maturation of most organ systems and comparable hemodynamics pattern compared to the human fetuses, serve as a good animal model for studying pregnancy and testing the effect of interventions on the fetal circulation.
Figure 11. Blood flow distribution of the human fetal circulation. The blood flows were expressed as mean flows on the left and as mean percentages of the combined ventricular output on the right. AAo, ascending aorta; DA, ductus arteriosus; DAo, descending aorta; FO, foramen ovale; LA, left atrium; LV, left ventricle; MPA, main pulmonary artery; PBF, pulmonary blood flow; RA, right atrium; RV, right ventricle; SVC, superior vena cava; UA, umbilical artery; and UV, umbilical vein (Reproduced from Prsa et al., 2014).
Figure 12. Comparison of the blood flow distribution of the fetal circulation in sheep (left) and in humans (right). The blood flows were expressed as mean flows on the left and as mean percentages of the combined ventricular output on the right. AAo, ascending aorta; DA, ductus arteriosus; DAO, descending aorta; FO, foramen ovale; LA, left atrium; LV, left ventricle; MPA, main pulmonary artery; PBF, pulmonary blood flow; RA, right atrium; RV, right ventricle; SVC, superior vena cava; UA, umbilical artery; and UV, umbilical vein. The human fetal blood flows were obtained from Prsa et al. (2014) and the sheep fetal blood flows were obtained from Rudolph et al. (2009).

1.4.4 Effect of Acute Maternal Hyperoxygenation on Fetal Circulation

Ventilating maternal sheep with 100% oxygen and isoflurane is a standard protocol for sheep MRI experiments due to the requirement for restraining the sheep throughout the
scan and avoiding hypoxia. However, maternal ventilatory management may impact fetal circulatory physiology. The fetal pulmonary circulation is very sensitive to any changes in blood oxygen content. It is well-established that increased fetal oxygen saturations have a pulmonary vasodilatory effect in the sheep fetus (Heymann et al., 1969; Assali et al., 1968; Rudolph, 1979).

The biochemical mechanisms behind the resulting pulmonary vasodilation have been studied extensively. In the sheep fetus, vascular endothelial cells produce prostacyclin (PGI\(_2\)) which is a vasodilator acting through activating adenyl cyclase (Heymann, 1999). Advancing gestation leads to an increase in PGI\(_2\) production and therefore a decrease in pulmonary vascular resistance (Shaul et al., 1993). Binding of the endothelium-dependent vasodilators and stretch on the pulmonary vascular endothelium lead to stimulation of nitric oxide production (Black et al., 1997; Chang et al., 1992). Nitric oxide then stimulates the production of guanosine-3′-5′-cyclic monophosphate and thereby initiates a cascade leading to smooth muscle relaxation (Fiscus et al., 1988). The responses of the fetal pulmonary circulation to oxygenation are therefore likely to be modulated by the production of both PGI\(_2\) and endothelin-derived nitric oxide (Porayette et al., 2016; Shaul et al., 1993; Shaul et al., 1992; Tiktinsky & Morin, 1993).

In addition to an increase in pulmonary blood flow, Porayette et al. (2016) also identified a significant decrease in DA flow in late-gestational human fetuses as a result of acute maternal hyperoxygenation. This reduction in DA flow is likely due to the fact that with pulmonary vasodilation and increased pulmonary blood flow, less blood is available to be shunted from the main pulmonary artery through the DA. In the current study, we therefore anticipate that our ventilation protocol of the maternal sheep during the MRI scan will lead to increased pulmonary blood flow and decreased DA flow.
1.5 Summary

In summary, the fetal circulation is very different from the postnatal circulation because of the presence of the placental circulation and vascular shunts. Measuring and monitoring fetal circulatory distribution in the fetus may be particularly helpful for studying the fetal response to drug and mechanical intervention for various fetal disorders, such as chronic fetal hypoxemia. However, although each has unique advantages, the traditionally used techniques for measuring fetal blood flow, including electromagnetic and ultrasound flow probes, radionuclide-labeled microspheres and Doppler ultrasound have limitations that render them unsuitable for repeated measurements of fetal circulatory distribution over several hours to weeks throughout gestation to determine both acute and chronic changes. We therefore propose using PC-MRI, a potentially reliable and accurate way of measuring blood flow that is frequently employed in humans, as an alternative to existing methods and aim to establish its feasibility in the sheep fetus with blood pressure trigger for gating. However, we do anticipate that our blood flow measurement will deviate from those obtained from normal physiological states due to our protocol of anesthetizing and ventilating the ewe with 100% oxygen.
2 Research Objectives and Hypotheses

The primary objective of this study was to investigate the feasibility of PC-MRI for measuring blood flows in the major fetal vessels in the sheep. The major fetal vessels that we aimed to target included the AAo, MPA, SVC, RPA, LPA, DA, DAo, CCA, UV, and DV, in order to obtain a comprehensive understanding of the fetal circulation. We also aimed to calculate pulmonary blood flow (PBF), FO flow, and CVO. The flows obtained from these vessels have been compared with those obtained in previous studies, particularly Rudolph’s findings (1970), which were obtained under normal intrauterine conditions. Our secondary objective was to establish a preliminary baseline of PC-MRI blood flow measurements in the late-gestation sheep fetus under anesthesia and 100% oxygen ventilation for future interventional studies.

In keeping with our primary objective, we aim to test the following hypothesis:

PC-MRI is a feasible technique for measuring blood flows in major vessels of the late-gestational sheep fetus, with blood pressure triggers for gating.
3 Methods

The animals were housed in an animal facility in Adelaide, Australia upon arrival (Section 1). A fetal catheterization surgery was performed to allow for blood gas analysis and gating during MRI (Section 2). MRI scans were performed in the animal facility using a 1.5T scanner (Section 3.1, 3.2, 3.3). Post processing of the PC-MR images (Section 3.4) and statistical analysis (Section 4) were completed at the Hospital for Sick Children in Toronto. Post mortem analysis was performed in Australia (Section 3.5).

3.1 Animals

All experimental protocols were examined and approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute. The protocols followed the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes developed by the National Health and Medical Research Council. Ewes were synchronized and mated. At ~55 days gestation, ewes were scanned for confirmation of pregnancy and identification of the number of fetuses. Ewes carrying twin pregnancies were selected for inclusion in the study. 7 Merino ewes and their 13 fetuses were studied, including 10 fetuses from 6 twin pregnancies and 3 fetuses from a triplet pregnancy.

The ewes were housed in an indoor facility at the South Australian Health and Medical Research Institute. A constant ambient temperature of 20-22°C and a 12 h light/dark cycle were maintained in the facility. Each ewe was housed in an individual pen and was provided with ad libitum access to food and water. Each ewe was in view of other sheep. All investigators understood and followed the ethical principles outlined by Grundy (2015).
3.2 Surgical Procedures

At 120-121 days’ gestation (term, 150 days), we performed fetal catheterization surgery under aseptic conditions. General anesthesia was induced with diazepam (0.3mg/kg; intravenous) and ketamine (7mg/kg; intravenous). Anesthesia was maintained with the inhalation of isoflurane (1-2%) in 100% oxygen. Throughout the surgery, trained staff monitored and recorded the level of anesthesia and health status of the ewes every 15 minutes, including oxygen saturation, heart rate and end-tidal carbon dioxide. A catheter (Critchley Electrical Products, Silverwater, Australia) was inserted into the maternal jugular vein of the ewe.

The abdominal skin and muscles were then incised to expose the uterus and then the fetuses. We inserted vascular catheters into a fetal carotid artery (either left or right), a fetal jugular vein (either left or right) and the amniotic cavity as previously described (Morrison et al., 2002; Morrison et al., 2007; Wang et al., 2012). The fetus was returned to the uterus. The uterus was sutured closed. A small incision was made in the flank of the ewe, which allowed the exteriorization of the fetal catheters for future access. We then sutured the maternal abdominal muscle and skin in layers.

During surgery, antibiotics were administered to the ewe to prevent infection (153.5 mg of Procaine penicillin, 393 mg of benzathine penicillin, 500 mg of dihydrostreptomycin; Lyppards, Adelaide, Australia). Antibiotics were also administered to the fetus to prevent fetal infection (150 mg of Procaine penicillin, 112.5 mg of benzathine penicillin, 250 mg of dihydrostreptomycin; Lyppards). The ewes were then recovered from anesthesia, and given analgesia (20 ug/kg, Xylazil, Troy Laboratories, Australia).
3.2.1 Post-Surgical Care

Antibiotics were administered intramuscularly to the ewe for 3 days after the surgery. Antibiotics were administered to the amniotic sac of the fetus (500 mg of ampicillin; Lyppards) for 4 days after the surgery. The sheep were then allowed to recover for 3 days before MRI. The maternal and fetal blood gas status were monitored and recorded every day throughout the recovery period and after the MRI scan until post mortem.

3.3 MRI Scan

3.3.1 Fetal Gating

Fetal gating triggers were acquired using the fetal carotid catheters with a technique similar to that described by Yamamura et al. (2009). The fetal carotid and amniotic catheters were inserted during fetal surgery as described in the previous section. During the MRI scan, the catheters were connected to displacement transducers and a quad-bridge amplifier (ADInstruments, Castle Hill, Australia). The transducers and amplifier allowed the transmission of the fetal blood pressure and amniotic pressure. The transmitted data were sampled at a rate of 400 Hz and digitized using Chart 7 (ADInstruments, Castle Hill, Australia). The digitized data was sent to a computer and processed into blood pressure traces (LabChart 7, ADInstruments, Castle Hill, Australia). Thresholding of the blood pressure traces near the systolic peak blood pressure allowed the peaks to act as trigger pulses. The trigger pulses were then transmitted to the ECG unit of the MR scanner computer and served as a gating trigger for the PC-MRI.

The pressure traces were recorded in the software (LabChart 7), and later analyzed to calculate the fetal heart rate and blood pressure. Mean systolic blood pressure (SBP) and mean diastolic blood pressure (DBP) were derived and mean arterial pressure (MAP) was calculated as \[ \text{MAP} = \text{DBP} + 0.4 \times (\text{SBP} - \text{DBP}) \].
3.3.2 Imaging Protocols

Anesthesia was induced in the ewes, and maintained with the inhalation of 1-2% isoflurane in 100% oxygen for the duration of the MRI as required to restrain the ewe in the MR. All imaging was performed on a 1.5 T Siemens Sonata scanner (Erlangen, Germany). The maternal abdomen was located with localizer sequence. We then obtained scout images of the fetus in axial, sagittal and coronal orientations with a static steady-state free precession sequence. Figure 13 shows some examples of these survey images. Based on these images, we prescribed the imaging planes for the PC-MRI to align perpendicular to the long axis of the following vessels: descending aorta (DAo), ascending aorta (AAo), superior vena cava (SVC), main pulmonary artery (MPA), ductus arteriosus (DA), right pulmonary artery (RPA), left pulmonary artery (LPA), umbilical vein (UV), ductus venosus (DV) and a common carotid artery (CCA). The plane for image prescription for each vessel have been previously described in humans by Seed et al. (2014).

The PC-MRI was performed using the following sequence parameters: field of view, 240 x 320 mm; 20% phase oversampling; voxel size, 1.0 x 1.0 x 5.0 mm; repetition time, 7 ms; flip angle, 30°; 3 averages and 2 views per segment. A typical R-R interval of 400 ms resulted in a temporal resolution of around 28 ms, giving approximately 14 true cardiac phases. This was interpolated by the scanner into 20 calculated phases. The $V_{enc}$ was 150 cm/s for DAo, AAo, MPA, DA and CCA; 80 cm/s for RPA, LPA and UV; and 100 cm/s for DV. A typical scan time for each vessel was approximately 3 minutes, resulting in approximately 90 minutes’ total scan time for each fetus. Figure 14 shows an example of a single frame from a PC-MRI acquisition. We repeated PC-MRI acquisitions in the AAo in 4 fetuses and the acquisitions of the DAo were repeated in 2 fetuses to assess the reproducibility of our measurements.
Figure 13. Examples of steady state free precession anatomical survey images in three orthogonal planes to the fetal sheep fetus. The heart and major cardiac vessels can be visualized from these images. (A) Axial (B) Sagittal (C) Coronal. Arrows point to the heart.
Figure 14. An example of phase-contrast images obtained in the sheep fetus. The image was obtained axial to the fetus. The descending aorta was targeted. Arrows indicate the descending aorta on the velocity (left) and magnitude (right) images.

3.3.3 Maternal Position During the MRI

Initially, 4 ewes (with 8 fetuses) were placed supine in the scanner during the scan, so that the body coils could be placed as close to the fetus as possible for optimal signal. However, 2 of these ewes and their 4 fetuses experienced a decrease in blood pressure during or shortly after the scan, resulting in fetal demise. In retrospect, this was likely due to compression of the maternal uterine veins and IVC. The scans for these 4 fetuses were therefore excluded from the results and subsequent data analysis, as they were not representative of a normal physiological state. Subsequent ewes were placed in the scanner on their left side rather than their back to avoid uterine and vena caval compression.
3.3.4 Post-Processing

The blood flow in each vessel was measured by post-processing the PC-MR images with a commercial software (QFlow 5.6, Medis Medical Imaging Systems, Leiden, Netherlands). A region of interest was drawn around the vessel of interest (Figure 15). The blood flow in each vessel was indexed to the estimated fetal weight (Section 3.4). The waveform of each vessel was assessed to confirm the identity of the vessel, based on our prior experience in the human fetus. The MRI post-processing was repeated by the same observer to assess intraobserver variability. In order to assess the interobserver variability, the MRI post-processing was repeated by a second observer. The second observer was blinded to the first observer’s results.

Figure 15. An example of typical contours used to measure the flow in fetal sheep vessels. The red circles indicate the ascending aortic contour (QFlow 5.6, Medis, Leiden, the Netherlands).
3.4 Blood Gases

Fetal carotid arterial blood gas samples (0.5 ml) were collected daily to monitor fetal health with the measurement of PaO$_2$, PaCO$_2$, pH, oxygen saturation, haematocrit, hemoglobin and base excess temperature corrected at 39°C with an ABL 520 analyzer (Radiometer, Copenhagen, Denmark) calibrated for sheep blood. Blood gases were also obtained both immediately before the MRI in all fetuses, and during the MRI scan in a subset of fetuses.

3.5 Post Mortem Analysis and Estimation of the Fetal Weight

At 129-130 days gestation (6-8 days after the MRI), the sheep were humanely sacrificed with an overdose (8 g) of sodium pentobarbitone (Vibrac Australia, Peakhurst, Australia). The uterus was removed. The fetus was exteriorized and weighed. The fetal weight at the time of the MRI was estimated from the weight obtained at post mortem. A previously published equation for weight estimation derived from 74 sheep fetuses was used to calculate the weight at MRI (Edwards et al., 1999), which was used to index the PC-MRI blood flow measurements. The equation is as follows: fetal weight = 0.0008 $\times$ gestational age at post mortem - 0.1046 $\times$ gestational age at MRI + 3.6508, where fetal weight is in kg and gestational age is in days (Edwards et al., 1999). In one fetus, post mortem was not performed because it died 2 days before post mortem (6 days after MRI; sheep 2), but it was included in the analysis after careful evaluation of its blood gas data showing that it was healthy on the day of MRI until 6 days after MRI.

3.6 Statistical Analysis

Blood flow measurements are expressed as mean ± standard deviation. The CVO was calculated as the sum of MPA flow and AAo flow, with an additional 3% added to
account for coronary blood flow, based on prior microsphere findings (Rudolph, 2009). PBF was calculated as the sum of LPA and RPA flows. Flow across the FO was calculated as the difference between left ventricular output (CVO - MPA) and PBF. The percentage of blood flow measurements obtained successfully was calculated using the following equation: number of measurements obtained / (number of target measurements – number of target measurements with no acquisition attempted) × 100.

Reproducibility, intraobserver and interobserver variability of all blood flow measurements were assessed using Bland Altman, two-way random intra-class correlation (single measures) and Pearson’s correlation analyses. Intraobserver and interobserver variability of individual blood flow measurements for each vessel were assessed using Bland Altman analysis. Internal consistency of the blood flow measurements was assessed using Cronbach’s alpha and Bland Altman bias analysis. Fetal blood gases obtained before and during the MRI were compared using paired t test. The statistical analyses were performed using SPSS Statistics 5 (IBM, Armonk, USA) and GraphPad (Prism 6.0, San Diego, USA).
4 Results

4.1 Animal Outcomes

7 ewes and their 13 fetuses were studied, including 6 twin pregnancies and 1 triplet pregnancy. However, 2 ewes and their 4 fetuses died during or immediately after the MRI scan, and were therefore excluded from all analysis. The remaining 5 ewes and 9 fetuses were included in all analyses, including another fetus that died 2 days before post mortem (6 days after MRI; Sheep 2). It was included in the analysis after careful evaluation of its blood gas data showing that it was healthy on the day of MRI. Figure 16 shows a summary of the animal outcomes. The mean fetal gestational age at the time of the MRI scan was 123.0 ± 1.0 days, and at post mortem was 129.2 ± 0.8 days. The mean estimated fetal weight on the day of the MRI scan was 2.67 ± 0.40 kg. The mean fetal weight at post mortem was 3.03 ± 0.37 kg. The average fetal MAP (44.4 ± 10.6 mmHg) and heart rate (131 ± 11 beats per minute) were normal for fetuses at this gestational age throughout the recording period (Table 2).
Table 2. Average fetal heart rate and blood pressure throughout MRI.

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beats per minute)</th>
<th>Mean Arterial Pressure (mmHg)</th>
<th>Rate Pressure Product (mmHg x beats per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Hour</td>
<td>131 ± 12</td>
<td>43.3 ± 9.9</td>
<td>5654 ± 1443</td>
</tr>
<tr>
<td>Second Hour</td>
<td>131 ± 11</td>
<td>45.5 ± 11.4</td>
<td>6046 ± 1720</td>
</tr>
<tr>
<td>Average</td>
<td>131 ± 11</td>
<td>44.4 ± 10.6</td>
<td>5850 ± 1566</td>
</tr>
</tbody>
</table>

Figure 16. A summary of animal outcomes and the number of subjects included in each stage of the study. White boxes represent subjects included in data analysis. Black box represents subjects excluded from data analysis.
4.1.1 Fetal Blood Gases

Fetal blood gases on the day of MRI, prior to anesthesia, were within normal ranges for healthy fetuses (Table 3). Compared to the blood gases prior to anesthesia, the blood gases during the MRI showed significantly elevated blood $P_{O_2}$, increasing by $5 \pm 3$ mmHg from $26 \pm 2$ mmHg to $31 \pm 5$ mmHg ($P = 0.035$). The oxygen saturation during the MRI was significantly higher than before the MRI by $10.8 \pm 3.7 \%$ from $66.9 \pm 7.4 \%$ to $78.2 \pm 5.6 \%$ ($P = 0.010$). The pH was also significantly lower after the MRI started, which decreased by $0.10 \pm 0.02$, from $7.36 \pm 0.03$ to $7.27 \pm 0.02$ ($P = 0.001$). The $P_{CO_2}$, hematocrit, hemoglobin and base excess levels were not significantly different (Table 3).
Table 3. The blood gas status of the 9 fetuses prior to induction of anesthesia for MRI scanning (top) and during the MRI scan (bottom). The samples for blood gas analysis were obtained from the fetal carotid catheter. The means of the blood gas results from before the MRI and during the MRI were compared using paired t test.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Before or During MRI</th>
<th>P_{O2} (mmHg)</th>
<th>P_{CO2} (mmHg)</th>
<th>pH</th>
<th>Hemoglobin (g/dL)</th>
<th>Oxygen Saturation (%)</th>
<th>Base Excess (mEq)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before</td>
<td>27</td>
<td>43.2</td>
<td>7.37</td>
<td>9.4</td>
<td>73.5</td>
<td>-0.7</td>
<td>29.2</td>
</tr>
<tr>
<td>2</td>
<td>Before</td>
<td>29</td>
<td>43.3</td>
<td>7.32</td>
<td>7.5</td>
<td>78.3</td>
<td>-3.4</td>
<td>23.5</td>
</tr>
<tr>
<td>3</td>
<td>Before</td>
<td>28</td>
<td>48.2</td>
<td>7.36</td>
<td>9.3</td>
<td>69.7</td>
<td>1.2</td>
<td>28.9</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>37</td>
<td>52.4</td>
<td>7.25</td>
<td>8.1</td>
<td>83.6</td>
<td>-4.2</td>
<td>25.1</td>
</tr>
<tr>
<td>4</td>
<td>Before</td>
<td>28</td>
<td>45.7</td>
<td>7.39</td>
<td>9.5</td>
<td>72.0</td>
<td>2.2</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>31</td>
<td>45.2</td>
<td>7.3</td>
<td>8.2</td>
<td>81.4</td>
<td>-4</td>
<td>25.5</td>
</tr>
<tr>
<td>5</td>
<td>Before</td>
<td>26</td>
<td>51.3</td>
<td>7.38</td>
<td>9.6</td>
<td>63.9</td>
<td>4.5</td>
<td>29.7</td>
</tr>
<tr>
<td>6</td>
<td>Before</td>
<td>23</td>
<td>49.9</td>
<td>7.34</td>
<td>10.5</td>
<td>53.0</td>
<td>0.2</td>
<td>32.5</td>
</tr>
<tr>
<td>7</td>
<td>Before</td>
<td>23</td>
<td>49.2</td>
<td>7.34</td>
<td>8.0</td>
<td>63.4</td>
<td>1.9</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>29</td>
<td>60.4</td>
<td>7.26</td>
<td>7.5</td>
<td>76.9</td>
<td>-0.5</td>
<td>23.5</td>
</tr>
<tr>
<td>8</td>
<td>Before</td>
<td>23</td>
<td>46.1</td>
<td>7.36</td>
<td>7.7</td>
<td>64.8</td>
<td>0.3</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>26</td>
<td>64.6</td>
<td>7.25</td>
<td>8</td>
<td>71</td>
<td>0</td>
<td>24.9</td>
</tr>
<tr>
<td>9</td>
<td>Before</td>
<td>26</td>
<td>45.2</td>
<td>7.40</td>
<td>9.0</td>
<td>63.6</td>
<td>2.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>Before (n = 9)</td>
<td>26 ± 2</td>
<td>46.9 ± 2.9</td>
<td>7.36 ± 0.03</td>
<td>8.9 ± 1.0</td>
<td>66.9 ± 7.4</td>
<td>1.0 ± 2.3</td>
<td>27.8 ± 3.0</td>
</tr>
</tbody>
</table>
During (n = 4) 31 ± 5 55.7 ± 8.6 7.27 ± 0.02 8.0 ± 0.3 78.2 ± 5.6 -2.2 ± 2.2 24.8 ± 0.9

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>P</th>
<th>0.035</th>
<th>0.138</th>
<th>0.001</th>
<th>0.166</th>
<th>0.010</th>
<th>0.079</th>
<th>0.156</th>
</tr>
</thead>
</table>

*P* values were obtained using a paired *t* test, assessing the differences between the blood gas measurements obtained before the MRI and the blood gas measurements obtained during the MRI.
4.2 Blood Flow Measurements

We successfully measured blood flow in 82% of all the vessels in which acquisitions were performed, excluding the vessels for which no acquisition was attempted (Table 4; Figure 17). The percentage of blood flow measurements obtained successfully in each vessel, excluding the vessels for which no acquisition was attempted, is as follows: MPA, 100%; AAo, 78%; SVC, 100%; DA, 71%; RPA, 57%; LPA, 86%; DAo, 100%; UV, 100%; DV, 50%; CCA, 83%.

The blood flow measurements were expressed in ml/min/kg and as percentages of CVO (% of CVO). All measurements are presented in Table 4 and Figure 18. We found a mean CVO of 517 ± 104 ml/min/kg. The mean MPA flow was 266 ± 64 ml/min/kg or 51 ± 9 % of CVO. The mean DA flow was 200 ± 57 ml/min/kg or 37 ± 6 % of CVO. The mean AAo flow was 236 ± 64 ml/min/kg or 46 ± 9 % of CVO. We determined a mean UV flow of 212 ± 82 ml/min/kg; and a mean DV flow of 105 ± 59 ml/min/kg, which was equivalent to 50 ± 9 % of UV flow. The mean DAo flow was 352 ± 74 ml/min/kg or 64 ± 9 % of CVO; and the mean SVC flow was 121 ± 36 ml/min/kg or 23 ± 8 % of CVO. Only one CCA was visible from each MRI acquisition targeting the CCA due to the obstruction of flow with the catheter on the contralateral side. The mean CCA flow was 73 ± 22 ml/min or 17 ± 4 % of CVO. The mean flow across the FO was calculated to be 164 ± 20 ml/min/kg or 36 ± 11 % of CVO. The mean PBF was calculated to be 75 ± 37 ml/min/kg or 16 ± 5 % of CVO. The distribution is illustrated in Figure 19. Examples of the flow profiles were shown in Figure 20.
Table 4. Fetal blood flows in 9 late gestation sheep fetuses acquired by PC-MRI.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gestational Age at MRI (weeks)</th>
<th>Estimated Weight at MRI (kg)</th>
<th>Measured PC-MRI Flow (ml/min/kg)</th>
<th>Calculated PC-MRI Flow (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MPA</td>
<td>AAo</td>
<td>SVC</td>
</tr>
<tr>
<td>1</td>
<td>122</td>
<td>3.33</td>
<td>181</td>
<td>205</td>
</tr>
<tr>
<td>2</td>
<td>122</td>
<td>2.24</td>
<td>268</td>
<td>369</td>
</tr>
<tr>
<td>3</td>
<td>124</td>
<td>3.11</td>
<td>267</td>
<td>183</td>
</tr>
<tr>
<td>4</td>
<td>124</td>
<td>2.74</td>
<td>222</td>
<td>UQ</td>
</tr>
<tr>
<td>5</td>
<td>124</td>
<td>2.77</td>
<td>338</td>
<td>184</td>
</tr>
<tr>
<td>6</td>
<td>124</td>
<td>2.57</td>
<td>192</td>
<td>229</td>
</tr>
<tr>
<td>7</td>
<td>122</td>
<td>2.06</td>
<td>347</td>
<td>253</td>
</tr>
<tr>
<td>8</td>
<td>122</td>
<td>2.76</td>
<td>SF</td>
<td>232</td>
</tr>
<tr>
<td>9</td>
<td>123</td>
<td>2.49</td>
<td>312</td>
<td>UQ</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>9</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>123</td>
<td>2.67</td>
<td>266</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>1</td>
<td>0.4</td>
<td>64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gestational Age at MRI (weeks)</th>
<th>Estimated Weight at MRI (kg)</th>
<th>PC-MRI Flow (% of CVO)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MPA</td>
<td>AAo</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mean (%)</td>
<td></td>
<td>51</td>
<td>46</td>
</tr>
<tr>
<td>SD (%)</td>
<td></td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

NI, not identifiable from the acquisition due to inaccurate slice prescription. NA, no acquisition was attempted due to mistakes. SF, no acquisition was attempted due to scanner failure. UQ, unacceptable image quality. 1, fetal weight on the day of MRI was estimated using the following polynomial equation: Fetal weight = 0.0008 x gestational age at post mortem - 0.1046 x gestational age at MRI + 3.6508, where fetal weight is in kg and gestational age is in days (Edwards et al., 1999).
Figure 17. Examples of phase contrast images obtained in the sheep fetus. Phase contrast images were targeting descending aorta (top) and ascending aorta (bottom) in late-gestation sheep fetuses. Arrows indicate target vessels.
Figure 18. Box plots of fetal blood flows measured by PC-MRI. Measurements were obtained from 9 late gestational sheep fetuses. Blood flows were expressed in ml/min/kg (top) and percentages of the combined ventricular output (bottom). Each box shows the median and interquartile range (if ≥ 4 subjects); each whisker indicates ranges of blood flows. MPA, main pulmonary artery; A Ao, ascending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; DAo, descending aorta; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery; PBF, pulmonary blood flow; CVO, combined ventricular output; FO, foramen ovale.
Figure 19. Blood flows in the major vessels of the sheep fetus obtained by PC-MRI. The mean blood flows were expressed in ml/min/kg (left) and percentages of combined ventricular output (right). MPA, main pulmonary artery; AAo, ascending aorta; DAo, descending aorta; DA, ductus arteriosus; PBF, pulmonary blood flow; IVC, inferior vena cava; SVC, superior vena cava; RA, right atrium; FO, foramen ovale; LA, left atrium; RV, right ventricle; LV left ventricle; UA, umbilical artery; UV, umbilical vein.
Figure 20. Representative flow curves of targeted cardiac vessels measured by PC-MRI. AAo, ascending aorta; MPA, main pulmonary artery; DAo, descending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery.
Figure 20 (continued). Representative flow curves of targeted cardiac vessels measured by PC-MRI. AAo, ascending aorta; MPA, main pulmonary artery; DAo, descending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery.
4.2.1 Intraobserver Variability, Interobserver Variability and Reproducibility

An intraobserver correlation analysis of all blood flow measurements revealed excellent agreement with no significant bias ($R = 0.998, P < 0.0001; \text{bias} = -7.2 \text{ ml/min}; \text{SD of bias} = 21.5 \text{ ml/min}; \text{intra-class correlation coefficient (ICC) = 0.997}; \text{Figure 21}$). An intraobserver correlation analysis of each vessel showed the following results: AAo, bias = -27.9 ml/min, SD of bias = 17.1 ml/min; MPA, bias = 0.6 ml/min, SD of bias = 25.2 ml/min; DA, bias = 2.2 ml/min, SD of bias = 24.8 ml/min; SVC, bias = -3.1 ml, SD of bias = 17.3 ml/min; LPA, bias = 0.3 ml/min, SD of bias = 14.7 ml/min; RPA, bias = 1.8 ml/min, SD of bias = 12.8 ml/min; DAo, bias = bias = 21.0 ml/min, SD of bias = 10.6 ml/min; UV, bias = -8.1 ml/min, SD of bias = 17.2 ml/min; DV, bias = -10.3 ml/min, SD of bias = 16.7 ml/min; CCA, bias = 5.0 ml/min; SD of bias = 20.7 ml/min (Figure 21).

An interobserver correlation analysis of all blood flow measurements revealed excellent agreement with no significant bias ($R = 0.996, P < 0.0001; \text{bias} = -7.7 \text{ ml/min}; \text{SD of bias} = 26.2 \text{ ml/min}; \text{ICC = 0.996}; \text{Figure 22}$). An interobserver correlation analysis of each vessel showed the following results: AAo, bias = -0.6 ml/min, SD of bias = 26.5 ml/min; MPA, bias = -28.0 ml/min, SD of bias = 22.2; DA, bias = -8.8 ml/min, SD of bias = 46 ml/min; SVC, bias = -5.7 ml, SD of bias = 37.0 ml/min; LPA, bias = -14.3 ml/min, SD of bias = 19.0 ml/min; RPA, bias = -3.2 ml/min, SD of bias = 13.2 ml/min; DAo, bias = -6.5 ml/min; SD of bias = 24.5 ml/min; UV, bias = -1.7 ml/min, SD of bias = 6.0 ml/min; DV, bias = 10.7 ml/min; SD of bias = 29.2 ml/min; CCA, bias = 20.7 ml/min; SD of bias = 28.0 ml/min (Figure 22). Correlation analyses were not performed for examination of the intraobserver or interobserver variability of each vessel due to the small sample sizes in some vessels.

MRI measurements of the AAo were repeated in 4 fetuses and MRI measurements of the DAo were repeated in 2 fetuses. These showed a high degree of reproducibility and no significant bias ($R = 0.989, P = 0.0002; \text{bias} = -2.3 \text{ ml/min}; \text{SD of bias} = 26.5 \text{ ml/min}; \text{ICC = 0.990}$).
Figure 21. Intraobserver correlation between MRI flow measurements obtained by two different observers. Overall, good agreement between the two observers and no significant bias were found (top row; $R = 0.998$, $P < 0.0001$; bias = -7.2 ml/min; SD of bias = 21.5 ml/min). AAo, ascending aorta; MPA, main pulmonary artery; DAo, descending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery.
Figure 21 (continued). Intraobserver correlation between MRI flow measurements obtained by two different observers. Overall, good agreement between the two observers and no significant bias were found (top row; $R = 0.998$, $P < 0.0001$; bias = -7.2 ml/min; SD of bias = 21.5 ml/min). AAo, ascending aorta; MPA, main pulmonary artery; DAo, descending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery.
Figure 22. Interobserver correlation between MRI flow measurements obtained by two different observers. Overall, good agreement between the two observers and no significant bias were found (top row; \( R = 0.996, P < 0.0001; \) bias = -7.7 ml/min; SD of bias = 26.2 ml/min). AAo, ascending aorta; MPA, main pulmonary artery; DAo, descending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery.
Figure 22 (continued). Interobserver correlation between MRI flow measurements obtained by two different observers. Overall, good agreement between the two observers and no significant bias were found (top row; $R = 0.996$, $P < 0.0001$; bias = -7.7 ml/min; SD of bias = 26.2 ml/min). AAo, ascending aorta; MPA, main pulmonary artery; DAo, descending aorta; SVC, superior vena cava; DA, ductus arteriosus; RPA, right pulmonary artery; LPA, left pulmonary artery; UV, umbilical vein; DV, ductus venosus; CCA, common carotid artery.
4.2.2 Internal Consistency

Internal consistency of the flow measurements was evaluated in four different ways, each in a subset of fetuses based on the availability of blood flow measurements (Table 4). In a subset of 4 fetuses, an internal consistency assessment of the PC-MRI measurements was performed by the comparison of direct and indirect measurements of PBF (LPA + RPA versus MPA – DA, respectively; n = 3, Cronbach’s alpha = 0.214, bias = 13.0, SD of bias = 40.1). In another subset of 4 fetuses, an internal consistency assessment was achieved by comparing direct and indirect measurements of DAo flow (AAo + DA – SVC versus DAo, respectively; n = 4, Cronbach’s alpha = 0.950, bias = 34.0, SD of bias = 75.5). In another subset of 3 fetuses, internal consistency was assessed through comparing the cardiac output (AAo + MPA) and venous return (SVC + DAo + PBF; n = 3, Cronbach’s alpha = 0.984, bias = -30.7, SD of bias = 31.0). We also measured blood flow in the inferior vena cava (IVC) in 3 fetuses, and these were compared with the DAo measurements (n = 3, Cronbach’s alpha = 0.914, bias = -10.3, SD of bias = 65.2).

We did not assess the significance level of these correlations due to the small sample sizes. Neither did we perform internal consistency assessment in 4 of 9 fetuses due to a lack of blood flow measurements in certain vessels resulting from incorrect prescriptions of image planes or unacceptable image quality (Table 4). All internal consistency assessments revealed good agreement except the comparison of direct and indirect measurements of PBF. We attribute this to the small size of pulmonary arteries, which may make their measurements more prone to error.
5 Discussion

This study is the first to demonstrate the feasibility of PC-MRI for making flow measurements in multiple fetal vessels and thus assessing the circulatory distribution of the sheep fetus. We demonstrated good reproducibility, interobserver and intraobserver agreement, internal consistency and a high success rate for the target vessels. Furthermore, when the pulmonary vasodilatory effect of maternal hyperoxygenation was accounted for, our results were in agreement with prior reference data for the distribution of the fetal circulation. Compared to previously established techniques for quantifying fetal blood flows, PC-MRI has certain attractive features. It is relatively non-invasive (the only intervention being a well established technique for cannulating the fetal carotid artery) and allows for measurements to be made in a reasonably physiologic state. Perhaps most importantly, the method would allow for repeated measurements of multiple vessels over several hours to weeks throughout late gestation. PC-MRI might therefore be suitable for assessing both acute and chronic changes in circulatory physiology induced by interventions performed in the sheep model. This could be particularly helpful for studying pathologies or therapies for pregnancy disorders involving the fetal circulation such as intrauterine growth restriction and congenital heart disease. Moreover, herein, we established a preliminary baseline of blood flow measurements of PC-MRI in major fetal vessels in the late-gestation sheep fetus during maternal ventilation with 100% oxygen.

5.1 Comparisons with Previous Findings

Our blood flow findings were compared to the previous findings obtained using radionuclide-labeled microspheres and/or flow probes, which were collected under rather normal intrauterine condition without the administration of anesthetics or oxygen and therefore close to true values (Table 5, Figure 23). We found a mean CVO of 517 ± 104 ml/min/kg, which was close to previous findings of Rudolph et al. (1970; 527 ± 42 ml/min/kg) and Iwamoto et al. (1979; 474 ±35 ml/min/kg), determined using radionuclide-
labeled microspheres in 8 fetuses at 123-140 days’ gestation. The mean MPA flow (or the
right ventricular output) we determined was 266 ± 64 ml/min/kg or 51 ± 9 % of CVO; and
the mean DA flow was 200 ± 57 ml/min/kg or 37 ± 6 % of CVO. These values were much
lower than findings of Rudolph et al. (1970; MPA flow, ~66 % of CVO; DA flow, ~59 %
of CVO). By contrast, our mean AAo flow was 236 ± 64 ml/min/kg or 46 ± 9 % of CVO;
and mean PBF was 75 ± 37 ml/min/kg or 16 ± 5 % of CVO. These values were elevated
when compared to those determined using microspheres (AAo flow, ~31 % of CVO; PBF,
5.4 ± 1.2 % of CVO; Rudolph, 1970). Using ultrasonic flow probes, Wong et al. (1994)
determined a left pulmonary flow of 21.0 ± 17.5 ml/min/kg in 6 normal sheep fetuses of
around 136 days’ gestation. Similar to the microsphere findings, this left pulmonary blood
flow was lower than what we found (LPA flow, 36 ± 32 ml/min/kg).

We determined a mean UV flow of 212 ± 82 ml/min/kg; and a mean DV flow of
105 ± 59 ml/min/kg, which was equivalent to 50 ± 9 % of UV flow. The mean DAo flow
was 352 ± 74 ml/min/kg or 64 ± 9 % of CVO; and the mean SVC flow was 121 ± 36
ml/min/kg or 23 ± 8 % of CVO. Compared with the microsphere findings of Rudolph et al.
(1970, 2009), our blood flow measurements for the systemic circulation, characterized by
UV, DAo, SVC and DV flows, fell close to the reported ranges (UV, 213 ± 82 ml/min/kg;
IVC (which should be equal to DAo), 67.2 ± 1.9 % of CVO; SVC, 25.3 ± 1.5 % of CVO;
DV, 41 ± 6.1 % of UV flow; Table 5). Iwamoto et al. (1979) determined a similar
umbilical-placental flow of 190 ± 18 ml/min/kg in 8 sheep fetuses at 123-140 days’
gestation, using radionuclide-labeled microspheres. Similarly, Edelstone et al. (1978)
determined a UV flow of 230 ± 71 ml/min/kg in sheep fetuses of 116-134 days’ gestation
using microspheres, and found that ~53% of the UV flow went into DV, which was similar
to what we found (41 ± 6.1 % of UV flow). The proportion of UV flow that enters the DV
may vary significantly in sheep fetuses, ranging from 20% to 90% (Rudolph, 2009). The
mean flow across the FO was 164 ± 20 ml/min/kg or 36 ± 11 % of CVO, which was
slightly higher than the value reported by Rudolph et al. (2009) in his textbook (~30 % of
CVO) but close to the value determined by Anderson et al. (1985; 169 ± 54 ml/min/kg),
using microspheres in 8 sheep fetuses at ~135 days’ gestation.
However, our unindexed CCA flow (199 ± 76 ml/min) was higher than the values previously reported by van Bel et al. (1994) in sheep of 124-135 days’ gestation (left CCA, 43 ± 17 ml/min; right CCA, 46 ± 18 ml/min), which were obtained using ultrasonic flow probes and were not indexed to weight. Similarly, Bennet et al. (2007) determined a lower flow in the left CCA (~ 40 ml/min), using ultrasonic flow probes, in sheep fetuses of 103-104 days’ gestation.

5.1.1 Possible Explanations for Discrepancy in Carotid Flow

We speculated that the difference in CCA flow between our findings and previous findings may be partly due to the insertion of the carotid catheter. We inserted a catheter into a CCA (either left or right), and determined blood flow in the contralateral CCA using PC-MRI, whereas van Bel et al. (1994) and Bennet et al. (2007) did not. In sheep, there are two CCA’s (left and right) with roughly the same flow rate arising from two different origins. The left CCA arises from the aortic arch, and the right CCA arises from the brachiocephalic trunk. Obstructing the blood flow in one CCA with catheterization may lead to increased, compensatory flow in the contralateral CCA. However, Jellyman et al. (2005) determined a CCA flow of 72 ± 7ml/min in sheep of around 124 days’ gestation using ultrasonic flow probes, with the contralateral CCA also obstructed by a catheter as in our study. Although the value they determined appears elevated compared with those determined by van Bel et al. (1994) and Bennet et al. (2007), it is still lower than the CCA flow we determined (199 ± 76 ml/min). The reason for this difference between our findings and previous findings is unclear. We suspect that it may be in part due to the slightly elevated P\textsubscript{CO}_2 and decreased pH observed in the blood gases, indicating respiratory acidosis, which could lead to increased cerebral blood flow (Barker, 1966; Assali et al., 1966). Another possible explanation is the administration of isoflurane anesthesia during PC-MRI, which was not used in the flow probe technique. Isoflurane has been shown to increase cerebral blood flow by 50-100% in adult dogs (Gelman et al., 1984). The small size of the carotid artery may have also contributed to errors in measurements.
Table 5. A comparison of fetal PC-MRI blood flow measurements to those previously reported in the literature.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Previous Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age at MRI (days)</td>
<td>9</td>
<td>123</td>
<td>1</td>
<td>~ 121-140(^1), late gestation(^2), 124-135(^3)</td>
</tr>
<tr>
<td>Estimated Weight at MRI (kg)</td>
<td>9</td>
<td>2.67</td>
<td>0.4</td>
<td>~ 2401-3600(^1)</td>
</tr>
<tr>
<td>MPA (% of CVO)</td>
<td>6</td>
<td>51</td>
<td>9</td>
<td>~ 66(^2)</td>
</tr>
<tr>
<td>AAO (% of CVO)</td>
<td>6</td>
<td>46</td>
<td>9</td>
<td>~ 31(^2)</td>
</tr>
<tr>
<td>SVC (% of CVO)</td>
<td>6</td>
<td>23</td>
<td>8</td>
<td>25.3 ± 1.5(^1)</td>
</tr>
<tr>
<td>DA (% of CVO)</td>
<td>4</td>
<td>37</td>
<td>6</td>
<td>~ 59(^2)</td>
</tr>
<tr>
<td>DAo (% of CVO)</td>
<td>6</td>
<td>64</td>
<td>9</td>
<td>67.2 ± 1.9(^1) (IVC)</td>
</tr>
<tr>
<td>UV (ml/min/kg)</td>
<td>6</td>
<td>213</td>
<td>82</td>
<td>220 ± 20(^1)</td>
</tr>
<tr>
<td>DV (% of UV)</td>
<td>3</td>
<td>150</td>
<td>19</td>
<td>41.0 ± 6.1(^1, +)</td>
</tr>
<tr>
<td>CCA (ml/min)</td>
<td>4</td>
<td>199</td>
<td>476</td>
<td>~ 90(^3)</td>
</tr>
<tr>
<td>PBF (% of CVO)</td>
<td>3</td>
<td>16</td>
<td>5</td>
<td>5.4 ± 1.2(^1)</td>
</tr>
<tr>
<td>FO (% of CVO)</td>
<td>3</td>
<td>36</td>
<td>11</td>
<td>~ 30(^2)</td>
</tr>
<tr>
<td>CVO (ml/min/kg)</td>
<td>6</td>
<td>517</td>
<td>104</td>
<td>527 ± 42(^1)</td>
</tr>
<tr>
<td>DV (% UV)</td>
<td>3</td>
<td>50</td>
<td>19</td>
<td>41 ± 6.1(^1)</td>
</tr>
</tbody>
</table>

\(^1\), values were obtained from a microspheres study by Rudolph et al. (1970), in 7 sheep fetuses of 121-140 days gestation. \(^2\), values were not reported in the article describing the microsphere study and therefore obtained from the textbook summary of Rudolph et al. (2009) for sheep fetuses in late gestation. \(^3\), calculated
based on information in the textbook. Values were obtained from an ultrasound flow probe study of van Bel et al. (1994) for sheep fetuses of 124-135 days gestation.

Figure 23. A comparison of fetal PC-MRI blood flow measurements (left) to those previously reported in the literature (right). Values were obtained either from a microspheres study by Rudolph et al. (1970), in 7 sheep fetuses of 121-140 days gestation or from the textbook summary of Rudolph et al. (2009) for sheep fetuses in late gestation.

5.2 Possible Effect of Maternal Oxygen Administration

The left ventricular output (LVO) and the right ventricular output (RVO) we determined from AAo and MPA flows, respectively, showed a nearly equal left-right distribution (Table 5). However, Rudolph et al. (2009) reported that RVO is approximately 2/3 of the CVO and LVO is approximately 1/3 of the CVO in the normal sheep fetus.
We propose that this difference in findings is most likely attributable to the fact the ewes were ventilated with 100% oxygen during the scan, whereas Rudolph et al. did not anesthetize or ventilate the sheep during the administration of microspheres. In keeping with this hypothesis, the average oxygen saturation in the fetal carotid arterial blood measured during the scan was elevated by 10.8% compared with the pre-MRI value (Table 5). The average $P_{O_2}$ of the fetal carotid arterial blood was also elevated by 5.3 mmHg, from 26 ± 2 mmHg before to 31 ± 5 mmHg during the MRI. The mean carotid $P_{O_2}$ at the time of our blood flow measurements (31 ± 5 mmHg) was also higher than the $P_{O_2}$ that Rudolph et al. reported from the umbilical veins of their fetuses (28 ± 1 mmHg). Considering the umbilical vein $P_{O_2}$ is significantly higher than the carotid $P_{O_2}$ in the fetal circulation, we can conclude that the blood supplied to the pulmonary circulation in our experiment contained significantly more oxygen than in the experiments performed by Rudolph et al. (1970).

The pulmonary circulation is highly sensitive to changes in the blood oxygen content. Increases in blood oxygen content decrease pulmonary vascular resistance and vasodilate the pulmonary vessels. Increased oxygen level of fetal blood as a result of 100% maternal oxygen ventilation leads to vasodilation of the pulmonary circulation and thereby results in a three-fold increase in PBF (Konduri et al., 1993). This change in PBF is consistent with the difference in PBF that we observed compared with Rudolph’s results (Table 5). When PBF is elevated, pulmonary venous return to the left atrium also increases. This may explain the increase in AAo flow that we observed because increases in preload result in increases in ventricular output. The reduction in left ventricular afterload resulting from cerebral vasodilation secondary to hypercarbia and anesthesia may have also contributed to the increase in left ventricular output. As a result of the high proportion of RVO passing into the pulmonary circulation in our pulmonary vasodilated fetal subjects, a smaller proportion of RVO was available to enter the DA. This would potentially account for the lower DA flow we observed. Another possible explanation for the reduction in DA flow is that maternal oxygen administration may have caused DA
constriction (Rasanen et al., 1998; Tuzler et al., 1991). The DA constriction may then lead to increased right ventricular afterload and thus decreased RVO or MPA flow.

The differences we observed in circulatory distribution in sheep fetuses during maternal hyperoxygenation are consistent with previous findings. Previous studies on fetal lambs involving maternal hyperoxygenation have shown a reduction in pulmonary vascular resistance (Rudolph, 2011; Heymann & Rudolph, 1969; Assali et al., 1968). Rasenen et al. (1996) observed an increase in PBF and reduction in DA flow in human fetuses during maternal hyperoxygenation using ultrasound. Porayette et al. (2016) investigated the effect of acute maternal hyperoxygenation in the human fetal circulation using PC-MRI and discovered a significant increase in PBF and decrease in DA flow during maternal hyperoxygenation, which are consistent with our findings.

5.3 Advantages of PC-MRI in Comparison to Other Techniques

Several tools are currently available for measuring fetal blood flow, including electromagnetic flow probes, ultrasonic flow probes, microspheres, and Doppler ultrasound techniques. Each of these techniques have their unique strengths and limitations, giving rise to differences in applications.

Many groups have inserted electromagnetic and ultrasonic flow probes in fetal sheep to measure blood flow. These two methods are useful and reliable for assessing any acute changes in blood flow in response to interventions. However, they involve extensive surgical procedures, the mounting of a flow probe on every target vessel, anesthesia, and changes in fetal body temperature. Unlike PC-MRI or microspheres, flow probes are somewhat unsuitable for the comprehensive assessment of the distribution of the fetal circulation.
Rudolph and Heymann (1967) made use of the distribution of injected radionuclide-labeled microspheres across the circulation to quantify blood flow to each organ. This technique is performed without anesthesia several days post-surgery when neurohormonal control has returned to normal. It is therefore useful for determining blood flow under normal physiological conditions in the fetus. However, one limitation of the microsphere technique is that it only allows a limited number of measurements in each fetus. Monitoring of fetal blood flow over several hours are not feasible using this technique because fetal organs need to be processed to measure the radioactivity of microspheres in order to quantify blood flow. It is somewhat unsuitable for assessing the effect of interventions on the fetal circulation.

The major potential advantage of PC-MRI is the fact that it could allow for flexibility in timing of blood flow measurements compared to the microsphere technique. Monitoring blood flow fluctuations over several hours is possible with PC-MRI. With adequate care, indwelling fetal carotid catheters can be maintained for several weeks in the sheep fetus, so that both acute and chronic interventions and models of placental insufficiency or congenital heart disease would be amenable to investigation using our approach. Such an approach could open new avenues of research into the relationship between acute and chronic circulatory abnormalities and fetal organ growth and development. Further MRI techniques could be combined with PC-MRI to enhance this kind of assessment, including magnetic resonance oximetry and detailed morphologic and microstructural interrogation of fetal organs. Finally, if minimal disturbance to fetal physiology is desired, PC-MRI could potentially be performed by employing noninvasive fetal gating techniques, such as self-gating, ultrasound gating or metric-optimized gating techniques, thus avoiding fetal instrumentation altogether.

A third technique often used to determine fetal blood flow is Doppler ultrasound, an imaging technique that has many similarities with PC-MRI. A unique advantage of the Doppler ultrasound technique is that it does not require any invasive surgical procedures. However, the major pitfall of Doppler ultrasound with regard to the measurement of vessel blood flow is inaccuracy, which arises from a lack of precision intrinsic to the technique.
Unlike PC-MRI, Doppler ultrasound relies on the estimation of the cross-sectional area of a target fetal vessel while assuming a constant velocity over the entire vessel area. It has been shown in adult blood vessels that PC-MRI flow measurements were more accurate than Doppler ultrasound measurements (Lotz et al., 2002). However, both MRI and ultrasound also generally require anesthesia in order to successfully target fetal vessels in sheep, as the animals are not adequately cooperative when awake.

5.4 Limitations

A major limitation of employing PC-MRI in the sheep fetus is the requirement of anesthesia and ventilation with 100% oxygen during the scan in order to restrain the sheep. Isoflurane administration can decrease cardiac output, increase blood flow to the brain and the myocardium and decrease blood flow to the pancreas, stomach and portal system (Gelman et al., 1984; Frink et al., 1992), while maternal oxygen administration can result in blood flow re-distribution as described above (Section 5.2). We were therefore unable to obtain fetal blood flow measurements under normal physiological conditions. Moreover, we inserted a catheter into a CCA and measured the flow in the contra-lateral CCA. This may have further affected the physiological status of the CCA flow. Our results can therefore only serve as a preliminary baseline for future fetal sheep PC-MRI studies and should not be interpreted as a representation of normal fetal physiology. Although the anesthesia may have altered the normal physiological state, one enormous benefit of it was that the sheep fetus stayed still during the scan. This lack of fetal motion allowed for long scan times with excellent spatial and temporal resolution and eliminated the possibility of fetal motion artifacts. Furthermore, the primary objective of this study was not to determine fetal sheep blood flow measurements under normal intrauterine conditions, which have previously been established. Our aim was to investigate the feasibility of PC-MRI in the sheep fetus using pulse triggers and to determine if a sufficient number of
vessels could be measured to create a complete picture of fetal hemodynamics (Figure 4). We have shown that this is possible.

Another limitation of the study is the small sample size. This is partly due to the fact that flows from only two vessels were measured in 2 animals (sheep 4 and 8) due to scanner failure, which likely resulted from the MRI system overheating. The MRI system used in this experiment was more than 15 years old, and we believe that this kind of problem will not be encountered when more up-to-date equipment can be used. We also experienced difficulty prescribing appropriate imaging planes for certain vessels, including the DA (in sheep 3 and 5), RPA (in sheep 2, 3 and 5), LPA (in sheep 3), CCA (in sheep 3), and DV (in sheep 3, 5, and 6). This may have resulted in differences in fetal anatomy in the fetal sheep compared with the human cardiovascular system, in which we have more experience. In some fetuses, one or more of these vessels were unidentifiable in the images we obtained. We conclude that they were not prescribed the correct plane. We found it particularly difficult to identify and prescribe the appropriate imaging planes for small, curved or short fetal vessels including the DA, DV, RPA and LPA. While we were performing the study, we gradually became more familiar with the anatomy of the sheep fetus and were increasingly successful at identifying and prescribing the appropriate imaging planes for these vessels.

In addition to these limitations, there are several other factors that may have affected the accuracy of our blood flow measurements, including spatial resolution, particularly in the smaller fetal vessels. However, with diameters of 3-5 mm and an in-plane spatial resolution of 1 × 1 mm, even the fetal pulmonary arteries should have been amenable to accurate flow quantification using our approach. Despite these limitations, we were able to show that our technique is feasible in the sheep fetus and we are confident that we would be able to measure hemodynamic changes in sheep models of placental insufficiency, chronic hypoxemia or congenital heart disease.
5.5 Future Directions

5.5.1 Future Application of PC-MRI in the Sheep Fetus

This study has shown that measuring and monitoring blood flows is possible in the sheep fetus with PC-MRI as a reliable technique to repeatedly measure blood flow in a sheep fetus. With the availability of PC-MRI, the fetal response to drug and mechanical intervention for various fetal disorders could then be studied. A particularly interesting condition for potential future investigation is chronic fetal hypoxemia, which often occurs as a result of placental insufficiency, diminished placental perfusion and umbilical cord occlusion or compression (Pearce, 2006; Richardson et al., 1998; Poudel et al., 2015). Studies have shown that in chronic fetal hypoxemia, the redistribution of blood flows become less pronounced as the fetal requirement for oxygen becomes downregulated, contributing to the poor outcome (Pearce, 2006; Richardson et al., 1998; Poudel et al., 2015). Evaluating and understanding the changes in blood flow distribution using a sheep model could therefore play an important role in assessing the physiology and pathology of fetal hypoxemia. PC-MRI could also be useful in the assessment of various drug or mechanical therapies aimed at relieving fetal hypoxemia in the sheep model (Zhang et al., 2016; Poudel et al., 2015). One example of a potential intervention which might be worth investigating is sildenafil, which preliminary studies suggest may improve fetal oxygenation in the setting of placental disease (Satterfield et al., 2010; von Dadelszen et al., 2011; Stanley et al., 2012; Dilworth et al., 2013; Itani et al., 2016). Examining the effect of sildenafil on the fetal circulation in a fetal sheep models of chronic hypoxemia could help us understand the efficacy and mechanism of such interventions on the fetal circulation. PC-MRI may also be applied to study the effect of interventions on the fetal circulation in sheep models of congenital heart diseases and post-myocardial-infarction cardiac regeneration.

In addition, fetal PC-MRI has been used in humans in combination with a MRI fetal oximetry technique developed by our team (Portnoy et al., 2017; Sun et al., 2015; Zhu et al., 2016). MRI fetal oximetry allows the measurements of hemoglobin concentration,
oxygen saturation and thus blood oxygen content using a combination T1 and T2 mapping of major fetal vessels (Sun et al., 2015; Zhu et al., 2016; Portnoy et al., 2017). A combination of PC-MRI and MRI fetal oximetry allows the quantification of fetal oxygen delivery and consumption. Quantifying the oxygen delivery and consumption could be helpful to diagnose intrauterine growth restriction cases in which blood flow distribution have pseudo-normalized in response to chronic hypoxemia.

Traditionally, intrauterine growth restriction has been diagnosed using ultrasound measures of estimated fetal weight (Baschat et al., 2000; Figueras et al., 2009). This approach has been shown to be problematic for constitutionally small for gestational age fetuses (Baschat et al., 2000; Figueras et al., 2009; Boers et al., 2010), which do not have underlying pathology or adverse pregnancy outcomes, and might be delivered inappropriately early. Currently, many centres use umbilical artery or middle cerebral artery pulsatility index and waveforms determined by Doppler ultrasound to predict adverse pregnancy outcome associated with intrauterine growth restriction. However, studies have shown that these pulsatility indices do not predict perinatal parameters with high reliability and therefore do not serve as an ideal indicator of intrauterine growth restriction (Sovio et al., 2015; Oros et al., 2010). Another indicator of intrauterine growth restriction is the cerebroplacental ratio which relies on detecting the redistribution of circulation that is normally present in intrauterine growth restriction using ultrasound. Although studies have shown that the cerebroplacental ratio is an effective predictor for adverse pregnancy outcome, it may normalize near the end of pregnancy as the flow redistribution diminishes in response to chronic hypoxemia (Richardson & Bocking, 1998; Zhu et al., 2016). An effective predictor of adverse pregnancy outcome and diagnostic tool for intrauterine growth restriction with these chronic fetal adaptations is therefore lacking.

In a recent study of human fetuses, Zhu et al. (2016) suggested that measuring fetal oxygen delivery and consumption, using a combination of PC-MRI and fetal MRI oximetry, can be a reliable way of diagnosing intrauterine restriction even when the flow redistribution has diminished in response to chronic hypoxemia. However, the accuracy of this method of calculating fetal oxygen delivery and consumption has not been validated in
uterine, due to the difficulty in determining the true oxygen saturation of a human fetus. A potential application of PC-MRI in combination with the MRI oximetry technique in the sheep fetus would allow for the validation of this technique. Confirming the accuracy of this method in the sheep could potentially support its use as a diagnostic tool for intrauterine growth restriction and thereby improve clinical management of this condition. Zhu et al. (2016) also demonstrated that SVC flow measured by PC-MRI is a more reliable indicator of IUGR with better diagnostic accuracy compared to existing Doppler ultrasound parameters such as cerebroplacental ratio. This finding further supports the value of PC-MRI as a highly sensitive tool for detecting hemodynamic changes in the whole fetal circulation in response to chronic fetal hypoxemia.

Moreover, in this study, we demonstrated that PC-MRI is feasible in late gestation sheep fetuses using blood pressure measured from fetal catheters as gating triggers. The gating technique we employed was invasive and required the surgical insertion of fetal catheters. We used an invasive gating technique as it was considered likely to be more accurate, and the disturbance to normal intrauterine condition was considered minimal as the fetuses were given days to recover from surgery before the scan. However, in future studies, minimal surgical intervention may be desired to examine the effect of interventions of fetal physiology. In such cases, it would be possible to completely eliminate the need for invasive surgery by employing noninvasive gating techniques for PC-MRI blood flow measurements. Some examples of noninvasive gating triggers include ultrasound gating, self-gating, metric optimized gating, and etc. Studies have shown that these techniques do not significantly affect the accuracy of PC-MRI blood flow measurements (Jansz et al., 2010; Nieman et al. 2009; Yamamura et al., 2011; Feinberg et al., 2010; Yamamura et al., 2015; Kording et al., 2015; Schoennagel et al., 2014). Combining these noninvasive gating techniques with our finding of the feasibility of PC-MRI for measuring fetal circulatory distribution in the sheep fetus may allow future PC-MRI studies to be conducted in the fetal sheep in an entirely noninvasive manner.

Future PC-MRI scans of the sheep fetus should be performed with 21% oxygen ventilation and close monitoring of the blood gases to avoid hypoxia, so that baseline
measurements under a more normal intrauterine condition can be obtained. Furthermore, in this study, one carotid artery was cannulated so that the arterial pressure pulse could be used for gating. This could have affected CCA flow we determined. Future experiments should be performed with a different artery cannulated, such as the brachial or femoral artery, or an external gating technique in order to measure undisturbed CCA flow.

5.5.2 Maternal Hyperoxygenation as a Therapy

Our study, although initially designed to demonstrate the feasibility of PC-MRI, employed a protocol of 100% maternal oxygen ventilation, which likely altered blood flow distribution when compared to previous studies made under more normal fetal physiological conditions. Our findings confirmed the observed changes in the fetal circulation resulting from maternal hyperoxygenation.

Maternal hyperoxygenation has been proposed as a potential fetal therapy for intrauterine growth restrictions and congenital heart diseases. Although its efficacy in improving fetal outcome for intrauterine growth restrictions was shown to be limited, many groups have reported its potential use for treating congenital heart diseases. Maternal hyperoxygenation has been used as a diagnostic tool in fetuses with hypoplastic left heart syndrome. It allows for the identification of atrial septal restriction, in which the usual pulmonary vasodilation associated with increased pulmonary oxygen content is impaired which affects the usual pulmonary vasodilatory effect of increased pulmonary artery oxygen content (Szwast et al., 2010). Maternal hyperoxgenation was also shown to be able to improve the growth of the aortic arch in a fetus with aortic coarctation (Kohl, 2011). In fetuses with underdeveloped cardiac chambers, maternal hyperoxygenation has been shown to improve the dimensions of left-sided heart structures (Szwast et al., 2009; Borik et al., 2014; Kohl, 2010; Lara et al., 2016). Porayette et al. (2016) showed that maternal hyperoxygenation lead to an increase in UV oxygenation and PBF with no change in UV flow, which indicated a likely increase in fetal oxygen delivery and pulmonary
vasodilation. Other studies have suggested that the impaired fetal brain growth typical of congenital heart disease may be due to reductions in the oxygen content of blood supplied to the fetal brain (Donofrio et al., 2003; Sun et al., 2015). It is therefore interesting to hypothesize that chronic maternal hyperoxygenation could potentially lead to improved fetal heart, brain and lung development in fetuses with congenital heart disease. These hypotheses may be amenable to testing in fetal sheep models of congenital heart disease.

5.6 Conclusions

This study has successfully demonstrated the feasibility of PC-MRI in the sheep fetus for measuring blood flows of major fetal vessels, including MPA, DAo, AAo, SVC, RPA, LPA, DA, UV, DV, and CCA. However, the small size of certain vessels such as RPA, LPA and CCA may pose some challenges with regard to acquiring adequate image quality. We have provided a preliminary baseline of blood flow measurements in these vessels. We have also demonstrated that acute maternal hyperoxygenation was associated with a different distribution of blood flow compared with flow measurements obtained by Rudolph et al. (1970, 2009) using radionuclide-labeled microsphere. We found increased pulmonary blood flow, reduced ductus arteriosus flow and increased ascending aortic flow when compared to previous microsphere findings. Future studies may employ PC-MRI to monitor blood flow changes in response to interventions in sheep models for chronic hypoxemia and other fetal conditions. These measurements may be enhanced by other MRI techniques providing information about fetal oxygenation and fetal growth and development.
References


Figueras, F., Oros, D., Cruz-Martinez, R., Padilla, N., Hernandez-Andrade, E., & Botet, F. et al. (2009). Neurobehavior in Term, Small-for-Gestational Age Infants With Normal


Yamamura, J., Schnnagel, B., Tavares de Sousa, M., Much, C., Ueberle, F., Adam, G., & Kording, F. (2015). Fetal cardiac MRI and left ventricular function assessment using a


Copyright Statement

Copyrights of all figures previously published, cited and reproduced in this thesis belong to their respective, original owners.

Permissions have been obtained from the publishers to reproduce these figures in print and electronic formats for educational and non-profit use in this thesis.