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Can Sonographic Assessment of Pulmonary Vascular Reactivity Following Maternal Hyperoxygenation Predict Neonatal Pulmonary Hypertension

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Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation predict neonatal pulmonary hypertension?

Volume 1/1

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Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of Doctor of Philosophy, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Date 13th July 2020

Abbreviations

2D	Two-Dimensional
2,3-DPG	2,3-bisphosphoglycerate
3D	Three-Dimensional
‘a’	Pre-systolic Wave
AAS	Aneurysm of the Atrial Septum
ABCA3	Adenosine Tri-phosphate Binding Cassette Protein Member A3
AC	Abdominal Circumference
AJOG	American Journal of Obstetrics and Gynaecology
AoI	Aortic Isthmus
AT	Acceleration Time
AT:ET	Acceleration Time to Ejection Time Ratio
ATP	Adenosine Triphosphate
AVSD	Atrioventricular Septal Defect
BMFMS	British Maternal and Fetal Medicine Society
BMI	Body Mass Index
BP	Blood Pressure

BPD	Biparietal Diameter
bpm	Beats per minute
ºC	Degree Celsius
Ca^{2+}	Calcium
cAMP	Cyclic Adenosine Monophosphate
CaO_2	Arterial Oxygen Content
CBF	Coronary Blood Flow
CC	Chest Circumference
CDH	Congenital Diaphragmatic Hernia
cGMP	Cyclic Guanosine Monophosphate
CHD	Congenital Heart Disease
CHF	Congestive Heart Failure
CI	Cardiac Index
cm	Centimetres
cm/s	Centimetres per second
CO	Cardiac Output
CO_2	Carbon Dioxide
COX	Cyclooxygenase
CPR	Cerebro-placental Ratio
CRF	Case Report File
CRH	Corticotropin-Releasing Hormone
CRHR1	Corticotropin-Releasing Hormone Receptor 1

CRRHBP	Corticotropin-Releasing Hormone Binding Protein
CRL	Crown Rump Length
CS	Caesarean Section
CTA	Clinical Trial Agreement
CTG	Cardiotocograph
CV	Coefficient of Variation
D	Diastole
DBP	Diastolic Blood Pressure
DICOM	Digital Imaging and Communications in Medicine
DO_2	Oxygen Delivery
DV	Ductus Venosus
DVP	Deepest Vertical Pool
ECG	Electrocardiogram
ECMO	Extra Corporeal Membrane Oxygenation
EDV	End-Diastolic Velocity
EF	Ejection Fraction
EFW	Estimated Fetal Weight
EMA	European Medicines Agency
EMR	Electronic Medical Records
eNOS	Endothelial Nitric Oxide Synthase

ET	Ejection Time
ET1	Endothelin-1
ET-CO_2	End Tidal Cardon Dioxide
FDA	Food and Drugs Administration
FHR	Fetal Heart Rate
FiO_2	Fraction of Inspired Oxygen
FL	Femur Length
FO	Foramen Ovale
GA	Gestational Age
GCP	Good Clinical Practice
g/dL	Grams per decilitre
GDPR	General Data Protection Regulation
GE	General Electric
GTP	Guanosine Triphosphate
Hb	Haemoglobin
HbO_2	Oxyhaemoglobin
HC	Head Circumference
HIE	Hypoxic Ischaemic Encephalopathy
HPRA	Health Products and Regulatory Authority
HR	Heart Rate
Hz	Hertz
ICC	Intra-class Correlation Coefficient

ICH	International Conference on Harmonisation
IQR	Interquartile Range
iNO	Inhaled Nitric Oxide
iPIMS	Integrated Patient Management System
IUGR	Intrauterine Growth Restriction
IVC	Inferior Vena Cava
K^+	Potassium
kg	Kilogram
KHz	Kilohertz
L/min	Litres per minute
LA	Left Atrium
LAA	Longitudinal Aortic Arch
LDA	Longitudinal Ductal Arch
Ltd	Limited
LV	Left Ventricle
LVCO	Left Ventricular Cardiac Output
LVEDP	Left Ventricular End Diastolic Pressure
LVO	Left Ventricular Output
MAS	Meconium Aspiration Syndrome

MCA	Middle Cerebral Artery
MD	Mid Diastole
MH	Maternal Hyperoxygenation
MHz	Megahertz
mL	Millilitre
mmol/L	Milimoles per Litre
MRI	Magnetic Resonance Imaging
NADPH	Nicotinamide-Adenine-Dinucleotide Phosphate
NEC	Necrotising Enterocolitis
NICOM®	Non-invasive Cardiac Output Monitor
NICU	Neonatal Intensive Care Unit
NIHR	National Institute for Health Research
NO-cGMP	Nitric Oxide-Cyclic Guanosine Monophosphate
NOS	Nitric Oxide Synthase
nPAAT	Neonatal Pulmonary Artery Acceleration Time
NSAID	Non-Steroidal Anti-Inflammatory Drug
O_2	Oxygen

P50	Oxygen tension at which 50% of Hb is saturated with O_2
PA	Pulmonary Artery
PAAT	Pulmonary Artery Acceleration Time
$PaCO_2$	Partial Pressure of Carbon Dioxide
PAF	Platelet Activating Factor
PAH	Pulmonary Arterial Hypertension
PaO_2	Partial Pressure of Arterial Oxygen
PCWP	Pulmonary Capillary Wedge Pressure
PDA	Patent Ductus Arteriosus
PDE5	Phosphodiesterase-5
PFC	Persistent Fetal Circulation
PFO	Patent Foramen Ovale
PG	Prostaglandin
PGI_2	Prostacyclin
PH	Pulmonary Hypertension
pH	Potential Hydrogen
PI	Pulsatility Index
PIV	Pulsatility Index for the Vein
PLI	Preload Index
PO_2	Partial Pressure of Oxygen

PPHN	Persistent Pulmonary Hypertension of the Newborn
PPROM	Preterm Prelabour Rupture of Membranes
PSV	Peak Systolic Velocity
PVIV	Peak Velocity Index for the Vein
PVL	Periventricular Leukomalacia
PVR	Pulmonary Vascular Resistance
RA	Right Atrium
RCPI	Royal College of Physicians in Ireland
RCSI	Royal College of Surgeons in Ireland
RDS	Respiratory Distress Syndrome
REC	Research Ethics Committee
RfPB	Research for Patient Benefit
RhoA-ROK	Rho A-Rho Kinase
RI	Resistance Index
ROS	Reactive Oxygen Species
RV	Right Ventricle
RVET	Right Ventricular Ejection Time
RVO	Right Ventricular Output
RVOT	Right Ventricular Outflow Tract

SaO_2	Oxygen Saturation
SBP	Systolic Blood Pressure
SD	Standard Deviation
S/D	Systolic to End-diastolic Frequency Shift Ratio
SDP	Single Deepest Pocket
sGC	Soluble Guanylate Cyclase
SIV	Site Initiation Visit
SMFM	Society of Maternal Fetal Medicine
SO_2	Functional Oxyhaemoglobin Saturation
SP-B gene	Surfactant Protein B gene
SpO2	Pulse Oximetric Measurement of Arterial Oxygen Saturation
SSRI	Selective Serotonin Reuptake Inhibitor
SV	Stroke Volume
SVC	Superior Vena Cava
SVR	Systemic Vascular Resistance
TAmax	Time Averaged Maximum Velocity
TAV	Time Averaged Velocity
TPR	Total Peripheral Resistance
TR	Tricuspid Regurgitation

TTN	Transient Tachypnoea of the Newborn
TVI	Time Velocity Integral
UA	Umbilical Artery
UAD	Umbilical Artery Doppler
$UaPO_2$	Umbilical Arterial Partial Pressure of Oxygen
UK	United Kingdom
USA	United States of America
UV	Umbilical Vein
$UvPO_2$	Umbilical Venous Partial Pressure of Oxygen
VD	Vaginal Delivery
VSD	Ventricular Septal Defect
VTI	Velocity Time Integral
V/V	Volume to Volume

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Thesis Summary

Objective:

Persistent pulmonary hypertension of the newborn (PPHN) occurs in 0.5 to 7 per 1000 live births and can result in significant cardiovascular instability. It occurs when there is a failure of the normal circulatory transition during the early newborn period. Recent studies have demonstrated that the fetal pulmonary vasculature responds in utero to maternal hyperoxygenation (MH). Maternal hyperoxygenation is used in an attempt to mimic the pulmonary vascular changes that occur at birth, when the neonate is required to take its first breath. The aim of this thesis work was to evaluate the fetal response to MH and to assess if fetal reactivity to MH would identify fetuses that would develop PPHN. Additionally, we aimed to ascertain the feasibility, acceptability and safety of performing the hyperoxygenation test in pregnant women.

Study Design:

Forty-six women with a singleton pregnancy greater than or equal to 31 weeks' gestational age were prospectively recruited to the study. Participants were selected and grouped as either, at risk of PPHN or as a study control. A fetal echocardiogram was performed on all subjects. A multivessel fetal Doppler examination was performed on the following vessels: the fetal pulmonary artery (PA), ductus arteriosus (DA), aortic

isthmus (AoI), ductus venosus (DV), umbilical artery (UA) and middle cerebral artery (MCA). Pulsatility index (PI), resistance index (RI), peak systolic (PSV) and end diastolic velocity (EDV), time-averaged velocity (TAV), acceleration time (AT), and ejection time (ET) were measured within the distal PA. The acceleration-to-ejection time ratio (AT:ET) was used to assess pulmonary vascular resistance (PVR). Doppler measurements were taken at baseline and repeated immediately following MH for 10 minutes (O_2 100% v/v inhalational gas) at a rate of 12L/min via a partial non-rebreather mask. Non-invasive cardiac output monitoring was undertaken for the duration of the hyperoxygenation exposure. Response to MH was considered where the decrease in PA PI was ≥10% from the baseline.

Postnatally, a comprehensive neonatal functional echocardiogram was performed within the first 24 hours of life to assess ejection fraction (EF), left ventricular output (LVO), and neonatal PA acceleration time (nPAAT). An additional twenty non-pregnant women were recruited to serve as controls for the evaluation of the safety of MH.

Results:

The median gestational age during fetal assessment was 35 [IQR 33–37] weeks. There was a decrease in fetal PA PI and PA RI following MH of 21% [IQR 9-36] from the baseline. There was an increase in the median fetal PA AT (43ms [40-47] to 57ms [47-60], p=0.005) leading to an increase in AT:ET

following MH (p=0.005), indicating a fall in PVR. No changes in the mean PIs of the UAD or MCA were observed following MH. There were no significant changes in the resistance indices of the DA. There was a significant increase in MCA blood flow, but not in MCA resistance indices. Fetuses that responded to hyperoxygenation were more likely to have a higher LVO ($p<0.01$) and EF ($p=0.03$) within the first 24 hours of life. These findings were not dependent on left ventricular size or mitral valve annular diameter but were related to an increased mitral valve inflow. There was no difference in nPAAT in the neonatal population. In the pregnant group there was a fall in maternal cardiac index (CI) during MH ($p=0.009$) coupled with a rise in systemic vascular resistance (SVR) with no recovery at ten minutes following the cessation of MH ($p=0.02$). In the non-pregnant group, there were no significant changes in any haemodynamic variable.

Conclusion:

Maternal hyperoxygenation offers the opportunity to assess the reactivity of the fetal pulmonary vasculature before birth. Our findings indicate a reduction in fetal PVR with a resultant increase in fetal pulmonary blood flow, increased left atrial return and increased LVO. This was not achieved at the expense of ductal constriction. There was evidence of improved MCA peak systolic velocity parameters; this was likely due to the positive impact of improved pulmonary venous return on left ventricular preload.

An increase in LVO and EF in the neonates who demonstrated a prenatal response to hyperoxia, suggests that the hyperoxygenation test can reflect functional rather than anatomical information in relation to the pulmonary arteries in utero. In our study, an appropriate response to hyperoxia in utero was also reflective of an optimal adaptation to postnatal life with rapid postnatal reduction in PVR increasing measured cardiac output. This warrants further exploration in a larger cohort to establish the ability of MH to predict the myocardial changes that occur during the transition to neonatal life.

We have demonstrated that MH is feasible and acceptable however, we have raised a concern in relation to the injudicious use of high flow oxygen in pregnancy. The haemodynamic changes observed in this study in response to MH during pregnancy could counteract any intended increase in oxygen delivery. The observed maternal effects of MH call for a re-evaluation of the role of hyperoxygenation treatment in the non-hypoxemic pregnant patient.

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Publications and Presentations

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Publications

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3. McHugh AF, Breatnach C, El-Khuffash A, Franklin O, Breathnach FM. 167: Maternal hyperoxia leads to changes in the fetal aortic isthmus Doppler waveform. American Journal of Obstetrics & Gynecology. 2020;222(1):S120.

4. McHugh A, El-Khuffash A, Bussmann N, Breatnach C, Tully E, Franklin O, Breathnach F. 418: Changes in fetal pulmonary artery

Doppler indices in response to maternal hyperoxygenation. American Journal of Obstetrics and Gynecology. 2019;220(1, Supplement):S283-S4.

5. McHugh A, Dwyer L, Breathnach C, Tully E, Franklin O, El-Khuffash A, Breathnach F. 96: Hyperoxygenation therapy in the third trimester leads to hemodynamic changes in maternal circulation. American Journal of Obstetrics and Gynecology. 2018;218(1, Supplement):S70.

6. McHugh A, Breathnach C, Dwyer L, Tully E, Franklin O, El-Khuffash A, Breathnach F. 244: The effect of maternal hyperoxygenation on fetal pulmonary vasoreactivity and on blood flow patterns in the umbilical artery and middle cerebral artery in pregnancy. American Journal of Obstetrics and Gynecology. 2018;218(1, Supplement):S158.

7. McHugh A, Dwyer L, Breatnach C, El-Khuffash A, Franklin O, Breathnach F. Changes in fetal pulmonary vasoreactivity in response to maternal hyperoxygenation. Fetal Medicine. BJOG: An International Journal of Obstetrics & Gynaecology. 2018;125(S2):4-5

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9. McHugh A, Breatnach C, Bussmann N, Franklin O, El-Khuffash A, Breathnach F. Do changes in fetal pulmonary vascular reactivity following maternal hyperoxygenation reflect the circulatory transition in the early neonatal period? Submitted to BMC Pregnancy and Childbirth. Accepted pending minor revisions.

Oral Presentations

1. A McHugh, A El-Khuffash, O Franklin, F Breathnach
   Hyperoxygenation in pregnancy exerts a more profound effect on hemodynamics than in a non-pregnant state
   Presented at Society of Maternal-Fetal Medicine (SMFM), 39th Annual Conference, Las Vegas, Nevada, February 2019

2. A McHugh, A El-Khuffash, O Franklin, F Breathnach.
   Hyperoxygenation therapy in the third trimester leads to hemodynamic changes in maternal circulation
   Presented at Society of Maternal-Fetal Medicine (SMFM), 38th Annual Conference, Dallas, Texas, February 2018

3. A McHugh, C Breatnach, A El-Khuffash, O Franklin, F Breathnach.
   Changes in fetal pulmonary vasoreactivity in response to maternal hyperoxygenation
   Presented at the British Maternal Fetal Medicine Society Conference (BMFMS) in Brighton, UK, April 2018

4. A McHugh, C Breathnach, O Franklin, A El-Khuffash, F Breathnach.
   Changes in pulmonary artery Doppler indices in response to maternal hyperoxygenation.

Presented at the Institute of Obstetrics and Gynaecology Annual Society Meeting in Dublin, Ireland, November 2018

Won first prize for best oral presentation

5. A McHugh, C Breathnach, O Franklin, A El-Khuffash, F Breathnach. The effect of maternal hyperoxygenation on fetal pulmonary vasoreactivity and on blood flow patterns in the umbilical artery and middle cerebral artery in pregnancy

   Presented at the Irish Perinatal Society Annual Meeting in Kilkenny, Ireland, November 2017

   Won first prize for best oral presentation

Poster Presentations

1. A McHugh, C Breatnach, A El-Khuffash, O Franklin, F Breathnach. Maternal hyperoxia leads to changes in the fetal aortic isthmus Doppler waveform
   Presented at the Society of Maternal-Fetal Medicine (SMFM), 40th Annual Conference, Dallas Texas, February 2020

2. McHugh A, El-Khuffash A, Bussmann N, Breatnach C, Tully E, Franklin O, Breathnach F. 418: Changes in fetal pulmonary artery Doppler indices in response to maternal hyperoxygenation.
   Presented at the Society of Maternal-Fetal Medicine (SMFM), 39th Annual Conference, Las Vegas, Nevada, February 2019

3. A McHugh, C Breatnach, O Franklin, A El-Khuffash, F Breathnach. Changes in maternal haemodynamics in response to hyperoxygenation in the third trimester.
   Presented at the British Maternal Fetal Medicine Society Conference (BMFMS) in Brighton, UK, April 2018

4. A McHugh, C Breathnach, O Franklin, A EL-Khuffash, F Breathnach. The effect of maternal hyperoxygenation on fetal pulmonary vasoreactivity and on blood flow patterns in the umbilical artery and middle cerebral artery in pregnancy.

Presented at the Society of Maternal-Fetal Medicine (SMFM), 38th Annual Conference, Dallas, Texas, February 2018

Prizes

1. A McHugh, C Breathnach, O Franklin, A El-Khuffash, F Breathnach. Changes in pulmonary artery Doppler indices in response to maternal hyperoxygenation.
   Presented at the Institute of Obstetrics and Gynaecology Annual Society Meeting in Dublin, Ireland, November 2018
   Won first prize for best oral presentation

2. A McHugh, C Breathnach, O Franklin, A El-Khuffash, F Breathnach. The effect of maternal hyperoxygenation on fetal pulmonary vasoreactivity and on blood flow patterns in the umbilical artery and middle cerebral artery in pregnancy
   Presented at the Irish Perinatal Society Annual Meeting in Kilkenny, Ireland, November 2017
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3. Awarded €26,500 funding from The Rotunda Foundation, for PhD research, through a competitive application process.

Dedication

Dedicated to Dave and Alanna

1 Chapter 1 Introduction

1.1 Background

Persistence of pulmonary hypertension leading to respiratory failure in the neonate is a condition that has been recognised for over half a century (1). It was originally described in 1969 as hypoxemia secondary to pulmonary vasospasm and subsequent right-to-left shunting of blood through the foramen ovale (FO) and/or ductus arteriosus (DA) and was named persistent fetal circulation or PFC syndrome (2). Now known as persistent pulmonary hypertension of the newborn (PPHN), this condition occurs in 0.5 to 7 per 1000 live births (3). It is a serious and often rapidly progressive disease that can result in significant cardiovascular instability in the newborn (4). Persistent pulmonary hypertension represents a failure of the normal circulatory transition during the early newborn period. A failure of the fall in pulmonary vascular resistance (PVR) results in pulmonary hypertension, reduced oxygen saturation and may result in right-to-left shunting of blood in the neonate (5). The shunting of blood occurs across persistent fetal channels, namely the patent ductus arteriosus (PDA) and the patent foramen ovale (PFO) (6). Some degree of pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure (7). The condition usually presents at birth or in the immediate newborn period.

There is a wide spectrum of severity in PPHN, from mild and transient increased oxygen dependence to severe hypoxemia and cardiopulmonary instability that may require intensive care management and support (8). It can lead to severe and potentially unresponsive hypoxemia and significant morbidity. The condition confers a mortality risk ranging from 4% to 33% (3, 9, 10). It can be associated with low systemic blood pressure (BP) and a low cardiac output (CO) due to increased right ventricular afterload, a reduction in left ventricular preload and myocardial dysfunction (11). Significant neurodevelopmental impairment at two years of age has been reported in approximately 25% of infants with PPHN (12-14). It is therefore of paramount importance that both the diagnosis and management of the condition are optimised.

Optimal management of PPHN remains controversial. The main objective in management is the treatment of the underlying condition and maintenance of adequate systemic BP. Enabling optimal alveolar ventilation and lung expansion by appropriate ventilator support, oxygen supplementation and mechanical ventilation all play a role in the management of PPHN (15). Pharmacologic interventions to increase pulmonary vasodilation and decrease PVR are crucially important. Oxygen remains the mainstay therapeutic intervention in management of the condition. However, overzealous application of oxygen therapy can result in oxygen toxicity. Therefore, the avoidance of hyperoxemia is equally as important as avoiding hypoxemia in PPHN management (16). In the treatment of term infants with

hypoxemic respiratory failure, maintaining arterial oxygen tension in the range of 6-10kPa, and oxygen saturations between 90%–97% is supported (17). Other treatments include nitric oxide (NO), exogenous surfactant therapy, phosphodiesterase inhibitors, prostaglandin analogs, prostacyclin (PGI_2), endothelin receptor antagonists and extracorporeal membrane oxygenation (ECMO) (6, 18). Inhaled nitric oxide (iNO) can improve oxygenation in 60%-70% of neonates with PPHN and its administration can significantly reduce the need for ECMO (19). However, despite the widespread use of iNO, the mortality and long-term morbidity for neonates with the condition remains unchanged (3). As many as 40% of infants treated with iNO will demonstrate either no improvement or a transient response to the medication (20, 21). Upon discharge from hospital, infants with PPHN warrant long-term follow up due to an increased risk of neurodevelopmental disabilities and chronic health conditions in the future (18). Long-term health problems such as chronic lung disease, hearing deficits, cerebral palsy and other neurodevelopmental disabilities can occur in approximately 14%-16% of survivors (15).

1.2 Aetiology of Persistent Pulmonary Hypertension of the Newborn

Persistent pulmonary hypertension of the newborn can be classified into three categories related to under development, mal-development, and mal-adaptation of the fetal lungs (22, 23). Putative aetiologic factors relating to

under development include congenital diaphragmatic hernia (CDH), pleural effusions, vascular anomalies, asphyxiating thoracic dystrophy and phrenic nerve agenesis (24). Severe lung hypoplasia secondary to oligohydramnios from preterm prelabour rupture of membranes (PPROM) or renal agenesis is another cause of PPHN (25). Pulmonary hypertension typically accompanies pulmonary hypoplasia when diminished surface area for gas exchange and inadequate pulmonary blood flow lead to hypoxia and remodeling of the resistant pulmonary arterioles (10). Many cases of PPHN are related to mal-development including chronic fetal hypoxia, fetal anaemia, and premature closure of the DA, as well as idiopathic or primary PPHN (22).

Idiopathic PPHN occurs in 10% of cases and in 1–2 per 1,000 live born infants (10, 26, 27). It occurs in the absence of any parenchymal disease or lung hypoplasia and is due to abnormal muscularisation of the pulmonary arterioles leading to severe hypoxemia with pulmonary vasoconstriction. Idiopathic PPHN is associated with polycythemia and hyperviscosity (8).

Most cases of PPHN are associated with parenchymal lung disease and are related to mal-adaptation including asphyxia, meconium aspiration syndrome (MAS), respiratory distress syndrome (RDS), sepsis, pneumonia and transient tachypnea of the newborn (TTN). Congenital heart disease (CHD) is also associated with PPHN and cardiac causes include total anomalous pulmonary venous return, left atrial, or mitral obstruction, and hypoplastic left heart syndrome (18). In addition, electrolyte disturbances such as hypoglycemia and hypocalcemia are associated with PPHN. Rarer causes

including alveolar capillary dysplasia (28), hyaline membrane disease caused by mutations in surfactant protein B (SP-B) gene (29) have been reported. Additionally, respiratory failure due to adenosine tri-phosphate (ATP) binding cassette protein member A3 (ABCA3) deficiency (30) has been described. Many of these rarer causes can result in an intractable and severe form of PPHN (6).

Studies of environmental risk factors have inferred a genetic susceptibility to PPHN (31, 32) due to the variation in the development and severity of disease (32-34). These include the use of non-steroidal anti-inflammatory drugs (NSAIDs) (34, 35) and selective serotonin reuptake inhibitors (SSRIs) during pregnancy. Non-steroidal anti-inflammatory drugs including ibuprofen, indomethacin, naproxen, and aspirin inhibit cyclooxygenase (36, 37). This results in a reduction or inhibition of arachidonic acid release which prevents the synthesis of prostaglandins and thromboxanes. These are important mediators in the regulation of the pulmonary vasculature and are involved in maintaining ductal patency (38). Serotonin increases fetal pulmonary vascular resistance (PVR) and the use of SSRIs in pregnancy has been associated with an increased incidence of PPHN (39). Maternal obesity, diabetes and asthma pose an increased risk to the newborn of developing PPHN (40-42). Genetic associations have been described such as genetic variants in corticotropin-releasing hormone (CRH), CRH receptor 1 (CRHR1) and CRH-binding protein (CRHBP) (31). To understand the condition, it is important to comprehend the differences between fetal and neonatal

circulations and the changes that need to occur during the transition from fetal and neonatal life.

1.3 The Fetal Circulation

The fetal circulation is characterised by a state of high PVR with reduced pulmonary blood flow (43). The metabolically active placenta consumes a significant proportion of the oxygen that is delivered to the gravid uterus via the uterine arteries. The oxygen content of the blood provided to the fetus is therefore lower than the maternal uterine arterial blood and thus results in the fetus living in a relatively hypoxemic environment (44). The placenta receives deoxygenated fetal blood via the umbilical arteries. Oxygen uptake occurs in the placental circulation and oxygenated blood returns to the fetus via the umbilical vein (UV). At the level of the fetal liver, the UV divides and about 50% of the blood flow is distributed to the left and right lobes of the liver. The remaining 50% passes through the ductus venosus (DV) (44). The Eustachian valve is a membranous valve which divides blood flow from the inferior vena cava (IVC) into two separate streams. It preferentially directs oxygenated blood from the DV through the FO to the left atrium (LA). In the second stream, de-oxygenated blood from the IVC passes through the tricuspid valve (45). Blood preferentially enters the right atrium (RA) from the inferior and superior vena cava. From the RA, blood flows into the right ventricle (RV). From there, the majority of blood is directed across the DA

into the descending thoracic aorta. Only 5%–25% of the right ventricular output (RVO) is directed to the pulmonary vasculature (7, 46). The fetal lungs do not participate in gas exchange during fetal life and pulmonary blood flow accounts for approximately 10% of the combined ventricular output in ovine fetuses (47). The placenta functions as the organ for gaseous exchange (48) and serves as the primary buffer, creating a large gradient between the maternal arterial partial pressure of oxygen (PO_2 = 90–100 mm Hg) and the UV (32–35 mm Hg) (49). This serves to limit high blood oxygen exposure to the fetus.

A high PVR is a normal state for the fetus and pulmonary vascular tone increases with advancing gestational age (GA) (4). Human fetal Doppler studies demonstrate a higher pulmonary blood flow (50) particularly as gestation advances. Higher pulmonary blood flow results in an increase in combined ventricular output from 13% at 20 weeks gestation during the canalicular stage of lung development, to 25% at 30 weeks gestation during the saccular stage of fetal lung development. The pulmonary blood flow then reduces to 21% at 38 weeks gestation (50). The fall to 21% closer to term is in response to active hypoxic pulmonary vasoconstriction secondary to the pulmonary vasculature developing a greater sensitivity to oxygen (50-52). The pulmonary arterial vascular impedance decreases during the second half of pregnancy until 34 to 35 weeks gestation (53). Despite ongoing lung growth after 34 to 35 weeks' gestation, the pulmonary vascular impedance thereafter remains unchanged (54). At ≥37 weeks gestation, pulmonary

blood flow increases substantially to almost half of the RVO (51). The descending aorta has an oxygen saturation of 60% and allows perfusion of the lower body and abdominal organs prior to returning to the low-resistance placenta (44). The upper part of the fetus including the brain, coronary arteries and the upper body receives blood exclusively from the left ventricle (LV) (55). The better oxygenated blood from the DV is mixed with a small volume of blood from the pulmonary veins before entering the ascending aorta to supply the carotid and coronary arteries, and therefore the brain and heart receive blood with a higher oxygen content of PO_2 = 25–28 mm Hg (49), and an oxygen saturation (SO_2) of 58%–65% versus 60% in the postductal aorta (44, 56).

The fetal systemic circulation is a low-pressure system, with right to left shunting of blood, high PVR and low systemic vascular resistance (SVR). A low oxygen tension environment exists in utero, which promotes high intrinsic myogenic tone and high vasocontractility (46). The elevated PVR in the fetal pulmonary arteries is related to its characteristic cuboidal endothelium which has a thick muscular coat (57, 58).

Pulmonary vascular tone remains persistently high in utero due to various vasoactive mediators such as endothelin-1 (ET_1), thromboxane, platelet activating factor (PAF), reactive oxygen species (ROS), and increased Rho A-Rho Kinase (RhoA-ROK) signaling (18, 46). The relatively hypoxic fetal environment also inhibits the production of vasodilators. However, with increasing GA there is an increase in vasodilator production. Endothelial

nitric oxide synthase (eNOS) and soluble guanylate cyclase (sGC) increase during late gestation (59). Increased expression of eNOS also occurs at birth and results in NO synthesis from l-arginine. The production of NO increases at the time of birth (60) and diffuses to the pulmonary smooth muscle cells to activate sGC. Cyclic guanosine monophosphate (cGMP) increases in response to increased sGC activity and results in smooth muscle relaxation. The enzymatic activity of phosphodiesterase 5 (PDE5) which converts cGMP to 5′ cGMP is inhibited by oxygenation (7).

1.4 The Neonatal Circulation

At birth, important changes in cardiac preload and afterload occur in quick succession (61). After the loss of the utero-placental circulation, there is a reduction in PVR, due to an increase in oxygen tension resulting in a ten-fold rise in pulmonary blood flow (46). There is a rapid involution of the medial smooth muscle and a thinning of the small pulmonary arteries which contributes to decreasing the PVR (57). The fall in PVR results in preferential flow of RVO through the pulmonary vascular bed instead of the DA. Neonatal survival is dependent upon a rapid, complex and well-orchestrated transition from the intra-to extrauterine environment (44).

Normal transition to newborn circulation requires high fetal pulmonary vascular pressures to fall, with dilatation of the pulmonary vessels. The initiation of breathing occurs spontaneously in most term and preterm infants

unless they have severe hypoxemia (62). Two minutes after birth, gas exchange is stabilised in most babies following a vaginal delivery. An improvement in the neonatal heart rate (HR) is the best clinical indicator of successful ventilation (44). Following the cessation of placental blood flow, there is a sudden increase in SVR, leading to an increase in LV afterload. Increased pulmonary blood flow results in an increase in pulmonary venous return to the LA and a resultant increase in LA pressure. When the pressure in the LA is greater than the right atrial pressure, the flap across the FO closes (63).

The FO and DA close to establish the postnatal circulation. Reversal to left to right blood flow across the DA occurs within 24 hours of birth in most term infants (64, 65). Pulmonary vascular resistance reaches adult levels by two weeks of age (46). In the new circulation, the LV ejects well oxygenated blood to the systemic arteries to supply oxygen and nutrients to all tissues in the body. Umbilical blood flow ceases and with the exception of a small amount of blood flow from the hepatic artery, all hepatic blood flow is derived from the portal vein (66). It is the removal of prostaglandins and the cessation of blood flow from the UV that contribute to closure of the DV by day six to ten of life (67). The left ventricular preload is entirely dependent on pulmonary venous return. The systemic venous system returns blood to the RA and RV to be pumped to the pulmonary arterial circulation. The right ventricular preload is dependent on adequate systemic venous return from the upper and lower body (61). From the RV, blood is oxygenated and

returns to the LA and LV via the pulmonary veins. The volume of blood ejected by each ventricle is similar and the arterial and venous circulations do not mix. The characteristic differences between the fetal and neonatal circulations are summarised in *Table 1.1*.

Table 1.1 Characteristics of Fetal Compared with Neonatal Circulation Physiology

Fetal	**Neonatal**
Right to left shunting PFO/PDA	Left to right shunting
Lungs do not partake in gas exchange	Lungs partake in gas exchange
Increased PVR	Decreased PVR
Decreased SVR	Increased SVR
Relative hypoxic environment	Normoxic environment
Limited ability to regulate stroke output (mostly via changes in heart rate)	Ability to regulate stroke output
Placenta	No placenta

Abbreviations: PFO, patent foramen ovale; PDA, patent ductus arteriosus; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance; Adapted from Morton et al (44).

1.5 Adaptations to Newborn Life

An improvement in the pulmonary blood flow as well as in ventilation and oxygenation occur after birth. This results in a decrease in PVR in newborn infants (18). A doubling of left ventricular output (LVO) occurs with a concomitant increase in stroke volume (SV) within the first hour after delivery (68). The LV, which in the fetus ejects about 150ml/min per kg, increases its output almost three-fold to 400–450ml/min per kg after birth. The increase in pulmonary blood flow facilitates gas exchange within the lung.

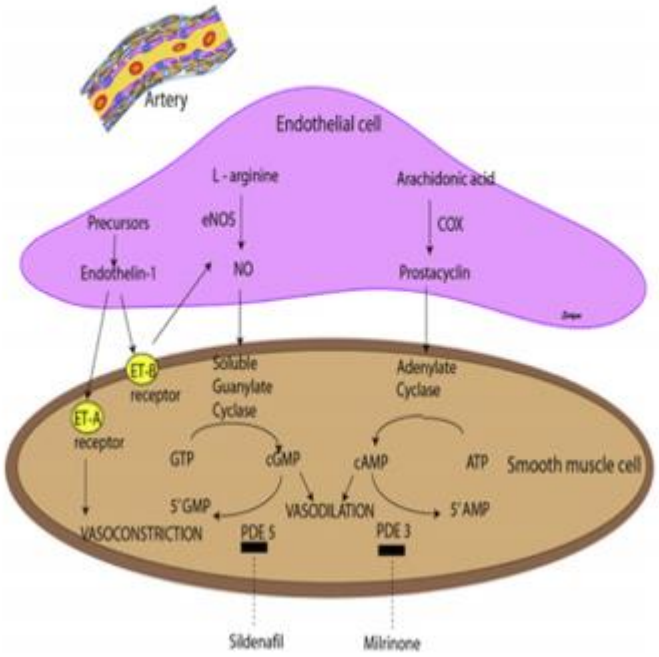
Physiologically, the pulmonary vasodilation that occurs in the newborn period is stimulated by the clearance of fluid from within the lungs, distention of the pulmonary air spaces and an increase in oxygen tension. The shear stress that is incurred from increased pulmonary blood flow is also involved in pulmonary vasodilation (60, 69, 70). However, the most important stimulus for pulmonary vasodilation is oxygen. A decrease in the partial pressure of carbon dioxide ($PaCO_2$) and an increase in pH also contribute to the response (71). Additionally, other active substances such as prostaglandins, bradykinins, and histamine may be involved in neonatal pulmonary vasodilation. This has been demonstrated in animal studies, where an increase in pulmonary blood flow independent of lung aeration has been noted in non-ventilated lungs (72).

The fetal to neonatal transition is regulated by many complex physiological and biochemical processes. Physiologically based umbilical cord clamping is an important component of a smooth transition. It ensures that LV preload,

oxygenation and BP are maintained thereby maintaining adequate LVO during the immediate neonatal period (73, 74). This practice has been shown to improve outcomes (75) in both preterm (76, 77) and term infants (78, 79). Nitric oxide also plays a central role in the transition, and oxygen stimulates NO release directly (80). Nitric oxide increases oxidative phosphorylation and results in a release of ATP from oxygenated fetal red blood cells (81). At birth, pulmonary blood flow increases eight to ten fold (82). Endothelial nitric oxide synthase which is predominantly expressed in endothelial cells, converts l-arginine and molecular oxygen to l-citrulline and NO. Endothelial nitric oxide synthase has also been detected in various other human cells including cardiac myocytes and platelets, specific neurons of the brain and in renal tubular epithelial cells (83, 84). Additionally, the isoenzyme has been identified in the syncytio-trophoblasts of the human placenta. To complete the process of converting l-arginine and molecular oxygen to l-citrulline and NO, eNOS utilises molecular oxygen and reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) as co-substrates (85). The vasodilator NO stimulates vasorelaxation by activating sGC to generate cGMP, while PDE5 impairs vasorelaxation by degrading cGMP.

Therefore, the normal transition is mediated by two pathways: the nitric oxide NO-cGMP pathway and the prostacyclin-cyclic adenosine monophosphate (c-AMP) pathway. Both pathways lead to pulmonary vasodilation through a decrease in intracellular calcium (Ca^{2+}) concentrations (15) (*Figure 1.1*).

Figure 1.1 Signaling Pathways that Regulate Fetal Pulmonary Vascular Tone



Signaling pathways that regulate pulmonary vascular tone in the developing fetal lung including: Endothelin-1, nitric oxide (NO) and prostacyclin (e.g. Prostaglandin I_2). Taken from (43).

Abbreviations: ET, endothelin; eNOS, endothelial nitric oxide; COX, cyclooxygenase; GTP, guanosine 5' triphosphate; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; ATP, adenosine triphosphate, PDE, phosphodiesterase.

1.6 Current Approaches to Neonatal Pulmonary Hypertension Diagnosis and Prediction

Accurate prenatal prediction of neonatal pulmonary hypertension would be an extremely useful clinical tool, as it would allow for advanced planning of delivery for those neonates thought to be at increased risk of the condition. Several methods have been proposed to assess fetal pulmonary vascular development including measurement of fetal chest circumference (CC) (86), chest area, chest area minus heart area, ratio of CC to abdominal circumference (AC) (87, 88), ratio of chest area to heart area and ratio of the chest area minus the heart area to the chest area (89, 90). Objective studies have included thoracic circumference to GA nomograms (91). Some of these methods are time consuming to perform and there can be considerable variability in measurements between different ultrasonographers. There are also additional limitations to these methods in assessing fetal pulmonary development. For example, the CC/AC ratio cannot be used in fetuses with a large AC, therefore fetuses with polycystic kidneys, obstructive uropathy or omphalocele would be excluded. Measuring the CC and CC/AC ratio has not been investigated widely in cases of prolonged rupture of membranes which account for many cases of pulmonary hypoplasia (92). Measurement of fetal thoracic biometry is insensitive and non-specific in predicting lung function (93).

Pulmonary hypoplasia as previously discussed is one of the causes of neonatal pulmonary hypertension. Efforts have been made to predict and

diagnose pulmonary hypoplasia prenatally, however this remains challenging (94). Studies applying three-dimensional (3D) ultrasound to estimate fetal lung volumes have calculated the lung volume by subtraction of the fetal heart volume from the thoracic volume. However, the resultant `lung' volume would not be a true reflection of the actual lung volume as other structures including the fetal thymus, various blood vessels and other mediastinal structures would not be accounted for in the measurement (95, 96). Magnetic resonance (MR) imaging has been applied in many studies investigating pulmonary hypoplasia (97, 98). However, despite the accuracy with which these modalities can estimate fetal lung volume, many of them cannot predict neonatal outcome (98).

Methods used to assess fetal lung maturity have been studied, including the lecithin to sphingomyelin ratio and the quantification of phosphatidylglycerol by thin-layer chromatography in amniotic fluid samples (99, 100). The amniotic fluid lamellar body count which represents a storage form of surfactant within the fetal pneumocytes has also been investigated for the purposes of assessing fetal lung maturity (101-103). However, all of these tests require an amniocentesis procedure to be performed, which carries an inherent risk of a pregnancy complication in approximately 0.7% of cases (104). More recent data has demonstrated a risk of miscarriage associated with amniocentesis of 0.3% (105). Risks include PPROM, placental abruption, fetomaternal haemorrhage (106), fetal injury and infection (107).

Previous studies have indicated that the capacity of the fetal pulmonary arteries to dilate can be judged prenatally, by administering high-dose oxygen to the mother (43, 90, 92). Recent studies have demonstrated that maternal hyperoxygenation (MH) induces vasoreactivity in the fetal pulmonary vascular bed (54, 94, 108). Following maternal oxygen therapy, a decrease in the PVR as demonstrated by fetal pulmonary artery Doppler, is deemed to indicate vasoreactivity in the pulmonary vasculature (109). Small studies to date indicate that a lack of vasoreactivity in response to MH, may serve as a useful clinical tool in predicting lethal pulmonary hypoplasia in at-risk fetuses (109, 110). The measurement of peripheral pulmonary velocity waveforms before and after MH may therefore help in determining the risk of developing PPHN. The ability to predict the fetal transition to neonatal life by a method that is non-invasive and reproducible would be beneficial for obstetric management, for parental counselling and for determining optimal neonatal management.

1.7 Hyperoxygenation in Pregnancy

Supplemental oxygen is administered to pregnant women in many different clinical scenarios in obstetric practice. It is often administered empirically, without any prior knowledge of maternal oxygen saturation. Maternal oxygen administration is commonly used in an attempt to improve fetal oxygenation (111-113). It is frequently administered in labour, in the setting of obstetric

emergencies and in an attempt to conserve fetal oxygenation in the operating room prior to Caesarean Section (CS) (114). Hyperoxygenation has been used more chronically in an attempt to improve fetal oxygenation in intrauterine growth-restricted (IUGR) fetuses (115) and it has been investigated as a potential diagnostic tool where a fetus is affected by a congenital cardiac abnormality (110, 116, 117).

Every year, over three million labouring women in the United States of America (USA) receive supplemental oxygen with the intention of improving the fetal metabolic milieu, but in the absence of evidence of maternal hypoxemia (118). Studies have shown that maternal oxygen administration does improve fetal oxygen levels and ameliorates fetal heart rate (FHR) patterns indicative of hypoxia (112, 119-122). However, there is no evidence that MH improves maternal or neonatal outcomes (123). Despite the widespread use of oxygen in intrauterine resuscitation, there is no clear guidance regarding indication for oxygen therapy, appropriate dose range, duration and curative effect (124).

The effects of hyperoxia have been well documented in the fetus (110, 116, 125-129). Experimental and clinical studies have shown that fetal pulmonary vasculature reacts to MH, especially in the latter stages of pregnancy (54, 71, 94, 108, 130-132). In sheep fetuses, changes in oxygen tension result in differing responses in the pulmonary vascular bed. Diminished fetal oxygenation has resulted in an increased fetal combined CO and pulmonary arterial vascular impedance (131), mainly due to an increase in weight-

indexed RVCO. However, severe acute hypoxemia in fetal ewes has resulted in no significant change in RVCO and combined CO. Increasing oxygen tension in fetal lambs increased pulmonary blood flow ten-fold in more mature fetuses and 0.2-fold in less mature fetuses (130). In human fetuses, an increase in fetal oxygenation leads to decreased PVR and increased pulmonary blood flow (54). Hyperoxygenation induces the release of several vasodilators including endothelium-derived NO and prostacyclin (PGI_2), resulting in a decrease in PVR and a concomitant increase in pulmonary blood flow (133). The vasoreactivity of the fetal pulmonary arteries has been attributed to the development of smooth muscle in the small fetal pulmonary arteries during the last trimester of pregnancy (46, 58, 134, 135). Oxygen is a critical substrate for NO synthesis. During MH, there is an increase in fetal oxygenation which activates NO synthesis (136). The release of eNO from the pulmonary vasculature is a Ca^{2+} dependent process (137). Endothelial potassium (K+) channels regulate intracellular Ca^{2+} flux and this contributes to the control of vascular tone in different fetal oxygen states (138). Increased NO release leads to pulmonary arterial vasodilatation in the fetus.

The fetal pulmonary circulation becomes more responsive to the vasodilator effect of oxygen with advancing GA. In human studies and in animal studies on fetal sheep, this response occurs after 31 weeks of gestation (54, 71). The reactivity of the fetal PA to changes in fetal oxygen tension can be detected by non-invasive pulmonary artery Doppler ultrasound techniques

between 31 and 36 weeks GA (54). Normative curves for the development of pulmonary reactivity induced by hyperoxia during gestation have been established (133). The measurement of pulmonary velocity waveforms before and after MH may therefore help in predicting how the fetus will adapt to the extra-uterine environment and transition to neonatal life.

There is a paucity of data describing changes in maternal haemodynamic indices to hyperoxygenation. A Cochrane review in 2003 of three small studies including 94 women, concluded that there is not enough data to estimate the benefits and risks of MH and that further trials of MH are warranted (115).

1.7.1 Oxygen

Oxygen is a colourless, odourless gas which is present in the atmosphere at 21% (139). Oxygen does not dissolve easily in blood and only a small amount is carried dissolved in the bloodstream (140). The “oxygen saturation level” describes the extent to which the circulating haemoglobin (Hb) is saturated with oxygen. On an arterial blood sample this is called the SaO2. On a pulse oximeter this is the SpO2 (141) (i.e the pulse oximetric measurement of arterial oxygen saturation). At partial pressures of arterial oxygen (PaO_2) that exceed 100mmHg the amount of dissolved oxygen will promptly increase (142). When the SaO2 is in the normal range of 94%-98% in a healthy adult it implies that almost all of the oxygen-carrying capacity of

Hb in the blood is being utilised. The administration of additional oxygen to a healthy person will therefore result in only a slight increase in the saturation level, from about 97% to 99% or to a maximum of 100% (140). Therefore, in this scenario there is only a very small increase in the amount of oxygen made available to the tissues. Increasing the fraction of inspired oxygen (FiO_2) increases oxygen transport. It does so by ensuring that blood Hb is fully saturated and by raising the quantity of oxygen that is normally carried in solution in the plasma (143). However, the solubility of oxygen in blood is low. The amount of oxygen delivered to the tissues in each minute is described as the global oxygen delivery (144, 145). It is a product of the CO and arterial oxygen content and is measured as:

Oxygen delivery = cardiac output × arterial oxygen content

$$DO2 = CO \times CaO2$$

Therefore, alterations in CO, arterial oxygen saturation, and Hb concentration will all effect oxygen delivery to tissues (144, 146). The oxygen content of blood is the volume of oxygen carried in each 100mls of blood. It is calculated as:

Arterial oxygen content=

(O_2 carried by Hb) + (O_2 in solution) =

$(1.34 \times Hb \times SpO_2 \times 0.01) + (0.023 \times PaO_2)$

1.7.2 Oxygen Use in Pregnancy

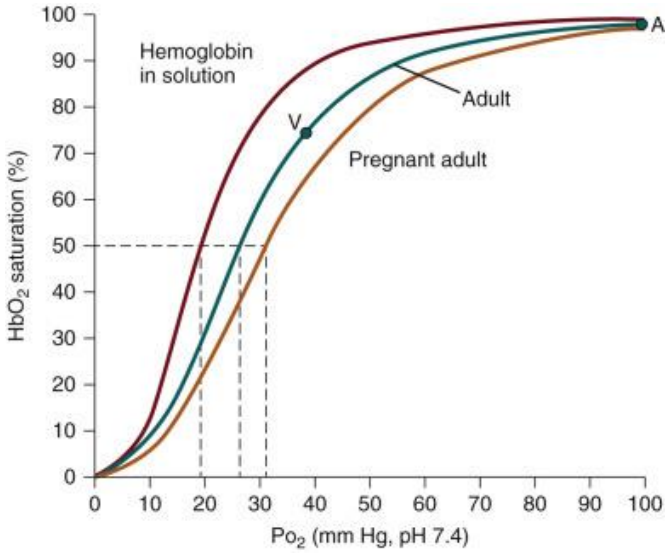
Hyperoxygenation in pregnancy with 50% oxygen significantly increases fetal partial oxygen tension (147). Fetal partial oxygen tension decreases in response to decreasing maternal inspiratory oxygen content in fetal sheep (131, 132). The same general principles apply for the use of oxygen during and outside of pregnancy. Oxygen is commonly given as part of the treatment for many obstetric emergencies (140). Oxygen administration during pregnancy or labour should be given by clinicians with the aim of achieving normoxemia (oxygen saturation 94%–98%). The same target range should be applied to women with hypoxemia due to acute complications of pregnancy for example collapse related to amniotic fluid embolus, eclampsia, antepartum or postpartum haemorrhage (114). Pregnant women with underlying hypoxemic conditions such as heart failure should be given supplemental oxygen during labour to achieve an oxygen saturation of 94%–98% unless they are at risk of hypercapnic respiratory failure. In this setting the target range reduces to 88%-92% (140).

There is no randomised trial evidence to suggest that maternal ‘‘hyperoxemia’’ as a result of MH is beneficial to the mother or fetus. A Cochrane review of low-quality evidence on the use of supplemental oxygen for CS during regional analgesia in healthy term pregnant women concluded that the use of supplemental oxygen was associated with higher maternal and neonatal oxygen levels. Measurements of maternal SpO_2, PaO_2, fetal umbilical arterial blood ($UaPO_2$) and fetal umbilical venous blood ($UvPO_2$) were increased during oxygen administration. Higher levels of oxygen free radicals were also recorded (148). In relation to the short-term clinical outcomes for neonates, as assessed by Apgar scores, the authors suggested that supplemental oxygen administration was neither beneficial nor harmful. There remains limited data regarding the risks and benefits of MH on mother, fetus and newborn.

1.7.3 Oxygen Affinity

The relationship in blood between oxygen saturation and partial pressure is described graphically by the sigmoidal shaped oxygen–haemoglobin dissociation curve in *Figure 1.2* (145).

Figure 1.2 Oxyhaemoglobin Dissociation Curve



Oxyhaemoglobin (HbO_2) dissociation curve under standard conditions for normal blood in pregnant and non-pregnant women.

Also shown is the dissociation curve for haemoglobin in solution.

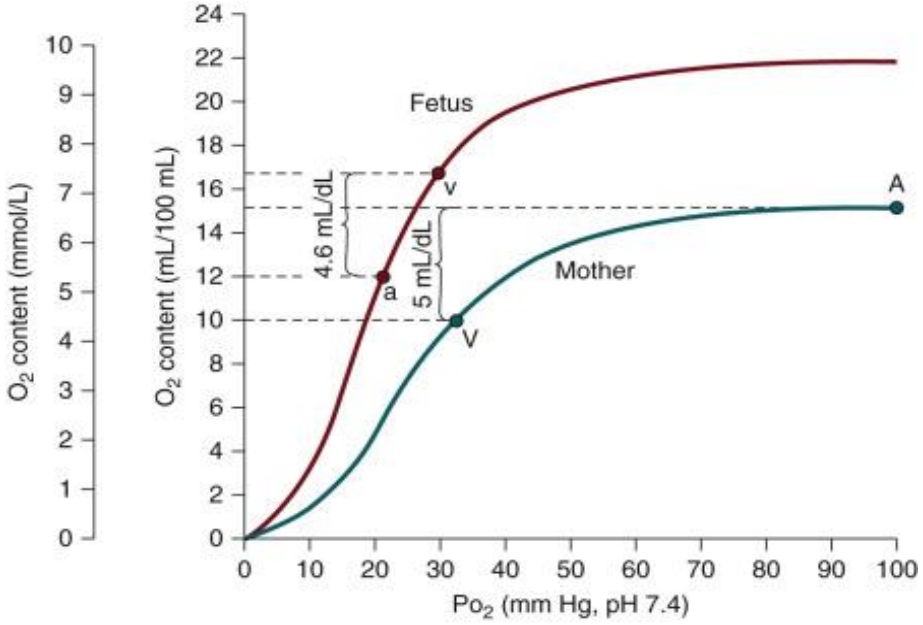
Copyright McNamara (61).

The increasing slope of the curve relates to the binding of oxygen to the Hb molecule at low levels of oxygenation. As all the Hb molecules approach full saturation at higher oxygenation levels, the shape of the curve flattens. The oxygen tension at which 50% of Hb is saturated with oxygen is expressed as P50 — that is under the criteria of a standard temperature of 37° C and a pH of 7.40.

The curve shifts to the left in conditions where the Hb oxygen affinity is increased or when the P50 is lower. Oxygen which is bound more tightly to Hb in this circumstance, is released only at lower partial pressures. The curve shifts to the right when the Hb oxygen affinity is decreased (i.e with a higher P50). Consequently, oxygen is bound less tightly to Hb and it is released at higher partial pressures (61). This enhances oxygen unloading at a tissue level. Therefore, the release of oxygen is dependent on the position of the oxygen equilibrium. A shift of the curve to the right is a normal finding in pregnancy (149). This is partly as a result of an increase in 2,3-bisphosphoglycerate (2,3-DPG) which is an intermediate of glycolysis. An increase in 2,3 DPG in pregnancy allows for an increase in oxygen delivery to the growing fetus. The extent of the shift to the right is directly related to the duration of pregnancy and increases as gestation advances (150).

Fetal blood has a higher affinity for oxygen (*Figure 1.3*). This affinity decreases as gestation advances and the HbO_2 equilibrium curve for the fetus progressively approximates that of the maternal HbO_2 equilibrium curve in advanced gestation (151, 152).

Figure 1.3 Oxyhaemoglobin Equilibrium Curve



Oxyhaemoglobin (HbO_2) equilibrium curve for blood from term infants is represented by the red curve.

HbO_2 equilibrium curve for blood from adult female mothers is represented by the blue curve.

Copyright McNamara (61).

1.7.4 Oxygen Delivery Systems

Oxygen is a drug and its administration requires careful monitoring. The delivery of excess oxygen is associated with significant complications (153, 154). Oxygen delivery systems are categorised into low-flow and high-flow systems (155). Lower oxygen flow than the actual inspiratory flow (approximately 30 L/min) is provided by low-flow systems. These include the nasal cannulae, the simple face mask, non-rebreather mask and a transtracheal oxygen catheter. With low-flow systems the oxygen is diluted with room air when the patient inspires. The degree of dilution will depend on the inspiratory flow pattern. These systems do not allow for an accurate calculation of the FiO_2. The FiO_2 represents the concentration of oxygen that a person inhales. Therefore, at a constant flow of oxygen, the larger the patient's respiratory tidal volume, the lower the FiO_2 and vice versa (156). High-flow oxygen delivery systems include a rebreather mask, venturi mask and high flow nasal cannulae. The FiO_2 remains stable with these delivery systems and it is not affected by the patient's type of breathing (155).

A non-rebreather mask delivers an oxygen concentration of between 60%-90% when used with a flow rate of 10-15L/min (157). The FiO_2 will depend on the patient's pattern of breathing and on the flow rate. A non-rebreather mask uses a reservoir bag (approximately 1000 mL) to deliver a higher concentration of oxygen (155). The mask has a one-way valve between the mask and the reservoir bag which prevents the patient from inhaling expired

air. If the oxygen flow rate is less than 10L/min the reservoir bag can collapse during inspiration (158).

Oxygen may be administered at concentrations of up to 100%; however, with most medical delivery systems, the actual inspired oxygen concentration will rarely exceed 60% (159). Lower oxygen concentrations should be administered to patients with chronic obstructive airway disease, as physiologically, they have a hypoxic drive for respiration. In the presence of high levels of oxygen, patients with chronic obstructive airway disease will under-ventilate their lungs leading to a respiratory acidosis and respiratory arrest in severe cases (143).

1.7.5 Adverse Effects of Oxygen

The pharmacology, pharmokinetics and toxicology of oxygen therapy have been well described (160). Potential adverse effects of supplemental oxygen therapy include parenchymal lung injury, airway injury and absorptive atelectasis (160, 161). However, these complications are reported in cases of long-term oxygen therapy where reactive oxygen intermediates can promote inflammation and induce cell death (162). Such adverse effects have not been demonstrated with the use of short duration oxygen therapy. There appears to be a threshold of 24 hours breathing an FiO_2 of 0.75 for producing signs and symptoms of oxygen toxicity in most humans (163). Symptoms can include substernal or pleuritic chest pain associated with

cough and progressive dyspnea (164). Breathing an FiO_2 of 0.55 or less for several days does not appear to produce signs of oxygen toxicity (163). Exposures to an FiO_2 of 0.60 for up to one week have not been proven to cause any specific lung injury. It has been reported that high flow concentrations of oxygen (FiO_2 >0.60) may damage the alveolar membrane when inhaled for more than 48 hours (143). Contraindications for the use of oxygen is in the presence of a naked flame and with active smoking, as it supports combustion (165). Facial burns and death of patients who smoke when using oxygen are well documented (166). There are no specific contraindications for the use of oxygen therapy during pregnancy and breast-feeding (140). Interactions with other medicinal products include amiodarone and bleomycin-induced lung disease (167, 168).

1.7.6 Maternal Effects of Oxygen Administration

Oxygen therapy has been investigated in pregnant patients in a multitude of settings and using varying dosages and regimens. In a prospective study of 62 pregnant participants, a mixture of room air and oxygen (100% at 9L/min) was administered via a face mask for ten minutes. The authors concluded that they did not observe any adverse maternal side effects during or immediately after oxygen administration (133), however maternal haemodynamics were not objectively assessed during the study. In another prospective study, 407 pregnant patients were studied over a 5-year period, 35 received long term MH for the investigation of severe early onset IUGR.

The mothers were hospitalised, rested in bed and given humidified oxygen to breathe via a medium concentration face mask at a rate of 8L/min (delivering a FiO_2 of about 0.55) continuously. In two of the 35 patients undergoing MH, therapy was interrupted for two days because of maternal hyperemesis. There were no other maternal complications reported (169). Similarly, in this study no objective assessment of maternal haemodynamics in response to the administration of oxygen was performed.

Simchem et al prospectively evaluated the effects of administering 100% oxygen and normocapnic hyperoxygenation to eight pregnant women in the third trimester. A CO_2 capnograph was used in the study to continuously monitor expired CO_2 levels, which were considered to approximate end-tidal CO_2 (ET-CO_2) levels. The authors described a decrease in adjusted ET-CO_2 by a mean of 12% from baseline when 100% oxygen was administered. This reverted to baseline during normocapnic hyperoxygenation (108). In the same study, minute ventilation increased by an average of 13% during hyperoxygenation. Maternal oxygen saturation did not change significantly in either phase of the study which included administration of 100% oxygen for 10-15 minutes (108). Although this study did incorporate an assessment of maternal HR, BP, oxygen saturation, ET-CO_2 levels and minute ventilation, the results are limited due to the small sample size of eight patients. In a prospective study of nine pregnant patients, the subjects received oxygen for either ten minutes at 8L/min (100% FiO_2) via a non-rebreather face mask or for greater than eight hours a day from 26 weeks gestation until delivery.

This particular study was investigating effects on aortic and mitral valve annular dimensions in fetuses with left heart hypoplasia. To standardise oxygen delivery in the study, a baseline maternal arterial blood gas sample was taken on the first day of chronic MH and the PaO_2 was measured. The blood gas sample was taken following a minimum period of one hour of supplemental oxygen administration. The authors found no significant maternal or fetal complications following acute or chronic MH administration apart from one subject (in the chronic MH cohort) who developed epistaxis on the first day of therapy. The authors concluded that there were no significant maternal or fetal complications to acute or chronic MH use (170). However, this study made no formal evaluation of maternal haemodynamics in response to MH. Brantberg et al prospectively investigated 25 singleton pregnancies where mothers received 100% oxygen at a flow rate of 8-10L/min. The authors concluded that there were no haemodynamic changes that suggested adverse effects related to oxygen administration (171), however no specific maternal haemodynamic indices were reported. The changes in relation to the fetal circulation that were reported in their study, echo those of others who have not demonstrated any deleterious effects on the fetus during maternal administration of 100% oxygen for periods of approximately 60-70 minutes (172, 173).

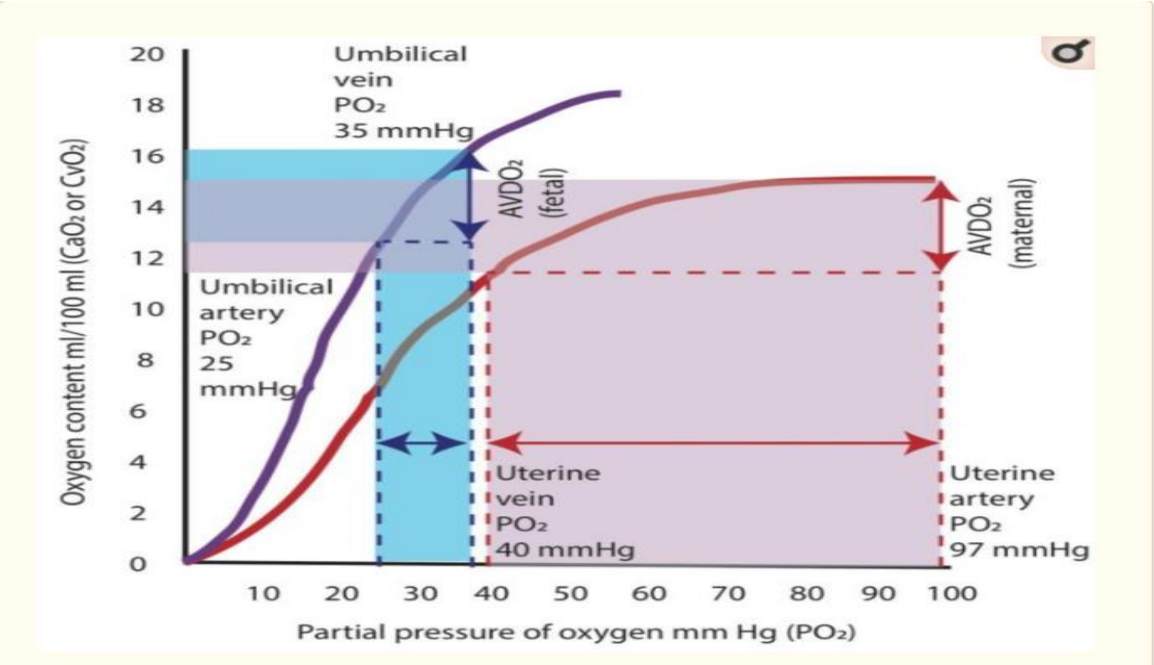
The authors of a prospective study of fifteen patients who were administered chronic intermittent MH in late gestation concluded that given its simplicity, universal availability and potential advantages for large numbers of patients,

comprehensive research at dedicated centres is required to ascertain the safety and appropriate dosing regimens for MH (125). Evidently, an accurate assessment of the maternal effects of hyperoxygenation are warranted if any such research is to be undertaken to define all suitable subjects for this novel therapeutic approach.

1.7.7 Fetal Effects of Oxygen Administration

The fetus can maintain a constant oxygen level even with a fall in Hb. If a pregnant women receives a transfusion of packed red blood cells, this can result in an increase of fetal PO_2 by approximately 5mmHg but the fetal oxygen content is completely maintained (174). This mechanism allows the fetus to avoid oxygen toxicity by keeping the oxygen tension in the blood low. By maintaining a constant oxygen content it allows an adequate delivery of oxygen to meet tissue demand (17) (*Figure 1.4*). The fetal Hb has a higher oxygen affinity and therefore shifts the oxygen dissociation curve to the left resulting in a greater release of oxygen at a lower PO_2 compared to adult Hb.

Figure 1.4 Oxygen Haemoglobin Dissociation Curves and Oxygen Supply



The purple curve= fetal Hb. The red curve = adult Hb.

A decrease in PO_2 from 97mmHg (level present in arterial blood) to 40mmHg (level in venous blood) in the adult, results in a release of oxygen amounting to approximately 5 mL/dL (area shaded in red).

For the fetus, the difference between the umbilical venous PO_2 (35 mm Hg) and the umbilical arterial PO_2 (25 mm Hg) results in a similar release of oxygen to the tissues of approximately 4mL/dL (area shaded in blue).

Abbreviations: $AVDO_2$: arterio-venous difference in oxygen content. Data from (17, 49) (Copyright Satyan Lakshminrusimha).

Experiments in fetal lambs have demonstrated that hypoxemia does not increase PVR at approximately 70% gestation (100 out of 147–150 days full term gestation), whereas at approximately 90% gestation (132–138 days) there is a doubling in the PVR (132). During hyperoxemia, a similar pattern is observed whereby a significant drop in PVR occurs in fetal lambs at a later gestation of 135 days, while at 94–101 days gestation no change in PVR occurs in response to increased oxygen tension (71, 130). The findings in these animal studies have been replicated in human studies where it has been demonstrated that the fetal pulmonary circulation is in a vasoconstrictive state until at least 31 to 36 weeks of gestation (54). Maternal hyperoxia does not alter fetal pulmonary blood at 20 and 26 weeks gestation, however there is an increase in pulmonary blood flow at 31–36 weeks gestation (54). The distribution of the RVCO has been shown to be affected by MH. However, the absolute fetal cardiac outputs themselves do not change (54). While fetal pulmonary arterial blood flow increases the volume of blood flow across the DA decreases (54, 138).

The effect of MH on left heart filling in fetuses with an aneurysm of the atrial septum (AAS) causing impediment to left ventricular inflow was investigated. The authors performed fetal echocardiography prior to and at 10 minutes of MH in 12 fetuses with an AAS and concluded that short term MH increases fetal pulmonary venous return. They described that MH substantially alters left ventricle geometry and promotes antegrade flow in the aortic isthmus (AoI) (175). This demonstrates proof of concept that MH can improve filling

of the left side of the fetal heart in fetuses with an AAS. They found that short term administration of MH was safe and reported no negative effects. Maternal hyperoxygenation has now become a standard diagnostic tool used in echocardiographic assessment of all fetuses with hypoplastic left heart syndrome at their institution in Philadelphia (175).

Short term administration of additional oxygen to pregnant women with pregnancies complicated by IUGR has been reported to give rise to a transient increase in fetal generalised movements, in fetal breathing movements and in fetal HR variation (176-179). A change in the distribution of the fetal CO and an improvement in cardiac oxygenation leading to increased CO and blood velocity in the descending aorta of fetuses with IUGR has also been described (169, 180). This indicates that the haemodynamics of the IUGR fetus can be positively affected as a result of MH.

1.7.8 Neonatal Effects of Oxygen Administration

The provision of ventilation with 100% oxygen for the neonate can promote the formation of ROS which can be damaging. Reactive oxygen metabolites such as superoxide anions appear to modulate pulmonary vascular changes and enhance vasoconstriction in the neonatal pulmonary circulation (181, 182). Inactivation of the valuable effects of NO can occur through the formation of peroxynitrite (183). Therefore, avoiding hyperoxemia and the generation of potentially damaging ROS is an important aspect in neonatal care. There is now data linking hyperoxygenation in infants with increased respiratory and neurological morbidity which has prompted the American Academy of Pediatrics to review its recommendations on neonatal resuscitation and oxygen administration (184, 185).

1.8 Maternal Adaptations to Normal Pregnancy

There are important physiological changes that occur in pregnant women that begin after conception and affect every organ system in the body (186). A combination of cardiac ventricular wall hypertrophy and an increase in end diastolic volume (secondary to an expanded blood volume), results in a net increase in the SV and subsequently in the maternal CO (CO= SV x HR) (187). Cardiac output increases during the first and second trimesters with the largest increase occurring by 16 weeks gestation (188). By eight weeks' gestation, the CO increases by 20% and at 16-20 weeks gestation it is 50%

above pre-pregnancy levels (189). The rise in CO then typically plateaus after 20 weeks' gestation but remains elevated until term. An increase in both the SV and HR are associated with the elevated CO. The increase in SV is as a result of an increase in preload, due to increased plasma volume and a decrease in SVR without any significant change in intrinsic cardiac ventricular contractility (190, 191). The maternal HR continues to rise until the third trimester when it is typically 10-15bpm greater than in the non-pregnant state. Despite a 20% to 30% increase in SV in early pregnancy, it declines towards term. The CO further increases during labour and delivery and then returns to a pre-pregnancy level by two weeks postpartum (189). The SVR progressively drops throughout pregnancy to a maximum of 30% below the non-pregnant baseline occurring in the early third trimester (192). The fall in SVR is due to peripheral vasodilatation which is mediated by endothelium-dependent factors, including NOS, upregulated by oestradiol and potentially vasodilatory prostaglandins (PG). Maternal position towards term can cause a marked effect on maternal haemodynamics. A fall in SV and CO can occur if a pregnant woman lies supine, given the aortocaval pressure effect of the gravid uterus on the IVC, reducing venous return to the heart. Therefore, pregnant patients should be nursed in the left or right lateral position or semi-recumbent position to reduce the risk of aortocaval compression. Like SVR, PVR also decreases significantly in normal pregnancy as does the serum colloid osmotic pressure which is reduced by 10%–15%. However, there is no increase in pulmonary capillary wedge

pressure (PCWP) (193) resulting in a colloid osmotic pressure/PCWP gradient reduction of about 30%. This results in pregnant women being particularly susceptible to pulmonary oedema. Mean pulmonary artery pressure also remains unchanged (194).

During pregnancy, there is a significant increase in the demand for oxygen owing to a 14% increase in the metabolic rate and a 20% increase in oxygen consumption (195, 196). Resting minute ventilation increases by 20%-50% of non-pregnant levels at term (191, 197, 198). There is a low expiratory reserve volume as a consequence of the cephalic displacement of the diaphragm (199). As a consequence of the increase in resting minute ventilation during pregnancy, the alveolar and arterial CO_2 tension (pCO_2) levels decrease to plateau around 27mmHg and 32mmHg, respectively (196, 200). This maternal hyperventilation causes arterial pO_2 to increase and arterial pCO_2 to fall. This is compensated for by a fall in serum bicarbonate to 18-22 mmol/L. Therefore, in pregnancy, a mild fully compensated respiratory alkalosis is normal, with a typical pH of 7.44 (201). Respiratory tidal volume levels increase as does arterial pO_2. The maternal oxygen reserve is reduced due to a decrease in the functional residual capacity combined with a rise in oxygen consumption (202).

1.9 Non-Invasive Cardiac Output Monitoring in Pregnancy

The ability to conduct non-invasive haemodynamic monitoring of pregnant patients has evolved in recent years (203-206). Maternal CO measurements using a pulmonary artery catheter and bolus thermodilution have been the clinical gold standard for central haemodynamic monitoring. Pulmonary artery catheter and bolus thermodilution measurements have been the reference standard used to compare newer non-invasive technologies (207, 208). However, it has the disadvantage of being invasive and carries the inherent risk of associated complications (209). Non-invasive monitoring of maternal haemodynamics is feasible, safe and cost effective (210-214). Maternal echocardiography has been utilised in studies evaluating CO in pregnancy (215-217). However, Doppler echocardiography is technically challenging, requiring a skilled operator, time-consuming to perform and the accuracy depends on the image quality obtained during the scan (209).

Transthoracic bioreactance is a new technique of non-invasive continuous CO monitoring based on the analysis of relative phase shifts of oscillating currents occurring when current traverses the thoracic cavity (218). The non-invasive CO monitor (NICOM®) uses this new technique of transthoracic bioreactance technology. It is performed by placing four dual sensors on the patient's chest in a manner that 'boxes' the heart. The NICOM® monitor sends a signal at 75kHz to the outer portion of the dual sensor and the known frequency is received via the inner portion of the dual sensor. A comparison in the frequencies is analysed by the machine. The NICOM®

observes the extent of the time delay or phase shift which has occurred. It determines approximately how much blood has exited the LV and entered the base of the aorta to cause the specific time delay. This measurement is then recorded as the stroke volume. The electrocardiogram (ECG) element of the NICOM® sensors detects the patient's HR and enables the machine to calculate the CO (CO = HR x SV). Synchronously, a BP cuff inflates to record the BP at intervals pre-designated by the operator. For accurate monitoring of CO in patients in a wide range of circulatory situations, the NICOM® system has proven to be acceptable and precise (212).

Clinical validation for the use of NICOM® in an obstetric population has been studied (219, 220). The NICOM® system has demonstrated repeatable measurements of SV and CO in pregnant women (219). Cardiac output measurements achieved through NICOM® have been shown to correlate with other non-invasive devices, in both normotensive and in hypertensive pregnant patients (221). Recent studies have demonstrated good agreement between NICOM® and 2D transthoracic echocardiography in estimating CO and SV, specifically in the third trimester of pregnancy (205, 222).

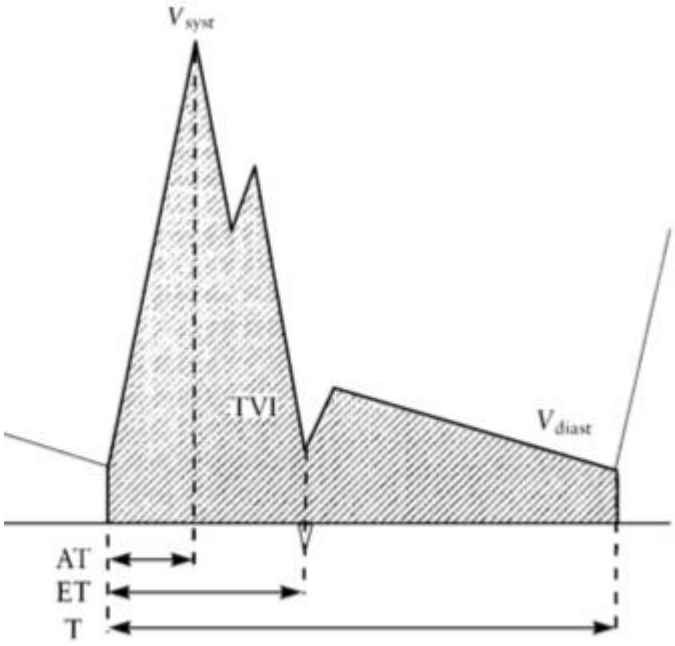
1.10 Fetal Doppler Measurements

1.10.1 Pulmonary Artery Doppler

The fetal pulmonary artery (PA) Doppler has a characteristic blood flow pattern at the proximal and middle branch level. The waveform has a rapid early systolic flow acceleration, a sharp mid-systolic deceleration, a deep notch or reverse flow in early diastole and a low diastolic forward flow (*Figure 1.5*) (223). The distal branches show a monophasic forward flow pattern with lower pulsatility and acceleration and deceleration velocities (223, 224).

The fetal PA acceleration time to ejection time ratio (AT:ET) can be easily measured (*Figure 1.5*) and it is has been correlated with advancing fetal GA (225) and fetal lung maturity testing in amniotic fluid (226, 227).

Figure 1.5 Pulmonary Artery Doppler Waveform



This figure illustrates the Doppler flow velocity waveform in the fetal pulmonary artery. This unique Doppler waveform pattern is characterised by a needle-shaped systolic peak. This peak is as a result of the rapid initial flow acceleration phase and an equally rapid deceleration phase. A more gradual decline in blood flow velocity then occurs, which is interrupted by a short reverse flow pattern at the beginning of diastole.

Abbreviations: AT, acceleration time; ET ejection time; T, Time; TVI, time velocity integral; Vsyst, ventricular systole; V diast, ventricular diastole.

Pulmonary artery AT:ET can predict the development of RDS in preterm infants (225, 228). Main PA AT:ET is inversely related to PA pressure measured directly at cardiac catheterisation in patients with congenital heart disease (229). Some studies have described that as the PA AT:ET increases the probability of fetal lung maturity decreases (226, 228). Others have demonstrated an inverse correlation between fetal PA AT:ET values and the diagnosis of RDS in the neonatal period (225, 227, 230). In fact, it has been recommend that a fetus with a main PA AT:ET below 0.305 has a risk of developing neonatal RDS and should be delivered in a hospital that has appropriate equipment and facilities to ensure optimal respiratory support in the neonatal period (101).

Fetal pulmonary vascular compliance increases as gestation advances and this coincides with a fall in mean PA pressure, resulting in a gradual increase in pulmonary blood flow (231). Continued pulmonary angiogenesis occurs as gestation advances in conjunction with an increase in the calibre of the lumen of the pulmonary vessels and an increase in vascular elasticity. This results in an inverse relationship between GA and the PA resistance index (RI) (232).

1.10.2 Aortic Isthmus Doppler

The fetal AoI functions as a connection between the cerebral and placental circulations. It plays an important role in fetal circulatory dynamics. Anatomically, it is situated at the origin of the left subclavian artery and the entry of the DA to the descending aorta (233). Obtaining the fetal AoI Doppler waveform measurement is feasible from either the longitudinal aortic arch (LAA) view or from the three vessel and tracheal view (234). Using a sagittal approach on ultrasonography, the aortic arch can be clearly visualised and the AoI Doppler can be obtained by placing the sample volume gate just beyond the left subclavian artery. This approach has been previously described (235).

There remains limited use of the AoI Doppler waveform in routine clinical practice. Analysis of the AoI waveform for monitoring fetal deterioration in IUGR fetuses has been described and it has been suggested that abnormal AoI Doppler resistance indices may represent a transition from hypoxemia due to placental insufficiency to cardiac decompensation (236). The AoI blood flow pattern has been investigated as a tool to predict perinatal outcome in growth restricted fetuses. It has been suggested that Doppler evaluation of the AoI is superior to the umbilical artery (UA) for the early detection of fetoplacental haemodynamic disturbances (237, 238). The merits of adding the AoI to the repertoire of Doppler investigations in fetuses with placental insufficiency have been highlighted and routine use of AoI Doppler velocimetry has been suggested in the evaluation of all growth

restricted fetuses (239). The volume and final systolic pattern of the AoI blood flow is determined by a myriad of competing physiological factors. These include the relative contributions of the left and right ventricular stroke volumes and by the ratio of the vascular resistances between the circulatory beds connected through the AoI (240-242).

1.10.3 Ductus Arteriosus Doppler

During embryogenesis, the aortic arch arteries three, four, and six persist and are re-patterned into the adult great arteries which include the common carotid, definitive aortic arch, and the DA (243). The DA provides a conduit between the main PA and the descending aorta. It originates near the origin of the left PA and terminates in the descending aorta distal to the left subclavian artery (67). In the fetal lamb, 90% of the right ventricular SV, which constitutes two thirds of the combined CO is shunted via the DA towards the descending aorta (244). The DA Doppler waveform is easily obtained and when scanning in a sagittal plane the ‘hockey stick’ appearance of the ductal arch is characteristic. Doppler flow velocity data from the DA has been recorded from as early as 11 weeks GA (245). Flow velocity in the DA is toward the aorta during both systole and diastole (246). Systolic flow velocity measurements in the DA, towards the descending aorta range from 65cm/s to a maximum of 140cm/s. During diastole the maximum flow velocity measurements in the DA range from 15cm/s to 35 cm/s (244). Gestational age does not result in any change in DA pulsatility

index (PI) values (244, 247). The DA exhibits increasing sensitivity towards prostaglandins from approximately 26 weeks of gestation (67, 248). Exposure to NSAIDs including indomethacin, a prostaglandin synthetase inhibitor, is associated with constriction of the DA. When constriction of the DA occurs, there is an increase in the peak blood systolic and diastolic blood velocities, which is more marked in diastole than in systole (248). The increased peak velocities results in a fall in the DA PI. The decrease in DA PI in mild cases is typically to a level below 1.9 and can be as low as 1.0 with associated tricuspid insufficiency in severe DA constriction (245, 246, 249). Constriction of the DA leads to an increase in pulmonary arterial pressure in the fetus, resulting in an increased development of the pulmonary arteriolar smooth muscle layer (250). The greater amount of smooth muscle may interfere with postnatal adaptation, producing a decrease in pulmonary arterial pressure that is slower than normal as a result of the higher PVR. Persistence of the high pulmonary arterial pressure delays normal maturation of the pulmonary vessels and the smooth muscle component can persist (67).

The fetal DA responds to varying oxygen tensions. While maternal hypoxemia has not been shown to alter DA PI values, maternal hyperoxemia results in an increase in measured DA PI values (138). The decrease in pulmonary arterial vascular impedance which occurs as a result of hyperoxygenation, especially during diastole, causes the increase in DA PI measurements. In normal circumstances, fetal PVR is higher than fetal SVR.

This allows for unidirectional shunting of blood across the DA toward the descending aorta (54). When the PVR decreases the forward blood flow across the DA also decreases, especially during diastole (54). Retrograde diastolic blood flow in the DA can occur if the PVR decreases below that of the SVR (138).

1.10.4 Umbilical Artery Doppler

From around eight weeks of gestation the fetoplacental circulation is established (251). Its development is characterized by a progressive rise in blood flow and a decrease in the vascular resistance to flow. It is a passive circulation in which the flow rate is determined by the mean effective perfusion pressure (252). The umbilical artery Doppler (UAD) has been used extensively for assessing downstream circulatory impedance or resistance to blood flow. Due to a lack of innervation of the umbilical blood vessels the UA tone is modulated by vasoactive substances that regulate the contractile process. Those that lead to contraction of the smooth muscle cells of the UA include serotonin, histamine, thromboxane, bradykinin, ET-1, and PGF2α (253, 254). Vasorelaxation is associated with activation of adenylyl and guanylyl cyclases, K^+ channels, and the inhibition of Ca^{2+} channels (253). Abnormalities in UAD waveforms are associated with pregnancy complications such as IUGR and preeclampsia (255). An increase in the impedance in the fetoplacental vascular bed results in a decline in the end-diastolic velocity (EDV) and an increase in Doppler indices such as PI, RI

and the peak systolic frequency shift to end-diastolic frequency shift (S/D) ratio. A S/D ratio >3.0 or a RI of >0.6 at greater than or equal to 28 weeks gestation is a valuable threshold for identifying pregnancies at high risk of adverse pregnancy outcome (256). Umbilical artery Doppler waveform shapes vary from those with present to absent or reversed EDV. Reversed EDV in the UAD is associated with an increased likelihood of a poor perinatal outcome if not acted upon (257, 258). An important prenatal determinant of perinatal outcome in growth restricted fetuses is an abnormal UAD measurement (259).

1.10.5 Middle Cerebral Artery Doppler

The blood flow resistance in the ascending aorta and the left ventricular afterload is predominantly determined by the brachiocephalic circulation (260).Therefore, changes in vascular resistance in the common carotid arteries reflect predominantly cerebral vascular blood flow resistance. The middle cerebral artery (MCA) Doppler waveform is easily obtained by performing fetal ultrasound. The vessel to sample is found with the colour or power Doppler setting on the ultrasound machine. The vessel is located overlying the anterior wing of the sphenoid bone near the base of the skull. The systolic velocity measurement of the MCA can decrease with distance from its point of origin. Therefore, the sample volume gate should be positioned as close as possible to the vessels origin in the internal carotid artery (261). In uncomplicated pregnancies, the MCA has a high resistance

flow and therefore minimal antegrade flow in fetal diastole (262). Measurement of the MCA PI and peak systolic velocity (PSV) are most commonly used in the investigation of IUGR and fetal anaemia respectively (263). Chronic fetal hypoxemia, as seen in IUGR elicits cerebral vasodilation with redistribution of the blood flow from the fetal periphery to the brain, this is known as the “brain-sparing effect” (264, 265). The MCA PI and RI values change throughout normal pregnancy and demonstrate a parabolic curve that plateaus between 28 and 30 weeks GA, likely due to the increased requirement of the brain during early and late pregnancy (266-269). Studies demonstrate a moderate positive correlation of MCA PI and MCA RI with GA up until 30 weeks of gestation. This is followed by a strong negative correlation with GA after 30 weeks gestation (263, 266, 269).

1.10.6 Ductus Venosus Doppler

The DV is involved in the distribution of highly oxygenated umbilical venous blood to the fetal heart (270). Its characteristic triphasic waveform pattern reflects the pressure-volume changes in the cardiac atria. The DV Doppler waveform is therefore a valuable tool in the assessment of any fetal condition that may affect forward cardiac function (271-273). Forward cardiac function can be influenced by various factors including ventricular afterload and preload as well as myocardial performance. The DV Doppler waveform can be obtained at the isthmus, near its origin from the UV in a mid-sagittal or cross-sectional abdominal plane (274). Fetal hypoxemia is

known to affect the DV waveform, causing an increase in DV diameter and in the pulsatility of the blood velocity waveform pattern. The waveform changes in response to hypoxemia are due to decreased forward blood flow velocity during atrial contraction ‘a-wave’ (275, 276). Fetal myocardial performance is related to decreased forward velocities during end-systolic relaxation represented by the ‘v-wave’ (270). As gestation advances and there is increased efficiency of forward cardiac function, the DV absolute velocity measurements increase significantly including the systolic, diastolic and a-wave blood flow velocities (270). An increase in absolute DV velocities results in a linear decrease in venous pulsatility and in a-wave-related ratios as gestation advances (277-279).

1.11 Summary

Sonographic assessment of the fetus during MH is an important diagnostic tool which may offer additional insights into the transition from the fetal to the neonatal circulation. If there is a failure of the normal circulatory transition in the early newborn period, persistence of high PVR may occur, resulting in pulmonary hypertension, low oxygen levels and marked right-to-left shunting of blood in the newborn heart (4). Approximately ten percent of neonates will require some form of clinical intervention at birth, with one percent requiring more extensive resuscitation (280). It is vitally important for clinicians to understand the changes that occur during the transition to neonatal life and to predict which neonates may have difficulty transitioning (280).

The haemodynamic and clinical consequences of pulmonary hypertension of the newborn may be similar between patients, however, it is important to recognise the potential for variability in the phenotypic presentation of the disease. The variability can occur due to the presence or absence of fetal shunts and in the ability of the myocardium to adapt to alterations in cardiac loading conditions (281).

Maternal oxygen administration has been used in an attempt to improve fetal oxygenation (123). It affects the distribution of fetal CO (169, 180) and PVR (237, 282). The use of MH for the investigation and treatment of fetal growth restriction has been evaluated (127, 283-285). Some effects of hyperoxia have been documented in the fetus (54, 170, 286). However, there is a

paucity of data describing changes in maternal haemodynamic indices to hyperoxygenation. A Cochrane review in 2003 of three studies including 94 women, concluded that there is not enough data to estimate the benefits and risks of MH and that further trials of MH are warranted (5).

Profiling the changes that occur in response to MH in fetuses may provide the opportunity to identify a unique profile associated with the development of PPHN. The ability to identify a cohort of fetuses with an increased risk of developing PPHN could enable tailored antenatal care improving decision-making in relation to the optimal site of delivery. Examining the changes that occur to maternal haemodynamics may allow us to have a better understanding of pregnancy physiology in the third trimester in situations where oxygen is administered.

In this PhD project, we studied the maternal, fetal and neonatal response to MH in utero. Using a clearly defined cohort of women with a singleton pregnancy in the third trimester, we prospectively evaluated alterations to maternal haemodynamics in addition to changes in fetal Doppler waveforms. Neonatal echocardiographic indices were measured in fetuses that responded to MH and in those that did not respond to MH.

The novelty and impact of this study is that the potential predictive role of MH has never been examined for the specific purpose of neonatal pulmonary hypertension prediction in at-risk and in low risk groups. In addition, this study explores an exciting and advancing area of fetal cardiology.

By undertaking this study, we have gained valuable information in relation to the feasibility, acceptability and safety of planning a future trial on the use of MH to predict fetuses that will develop neonatal pulmonary hypertension (PH). The issue of prenatal prediction of PH is important and relevant. The MH test would not add significantly to the cost of prenatal care and it may lead to a decrease in healthcare utilisation through better triage of these at-risk neonates. The study may impact our clinical management of these pregnancies in the future. The study may also impact the routine care of babies at risk of PH, in that; they may all undergo a fetal echocardiogram and MH test in the future as a means of risk stratification.

1.12 Study Hypothesis

The overall hypothesis of this thesis was that fetal pulmonary vascular reactivity to maternal hyperoxygenation, as assessed using PA Doppler ultrasound, could predict neonatal pulmonary hypertension.

As this was a pilot study we aimed to ascertain the feasibility, acceptability and safety of administering hyperoxygenation during pregnancy.

In order to evaluate our hypothesis we defined three primary and two secondary objectives as follows:

1.13 Study Objectives

Primary objectives

The primary study objectives were:

1. To assess feasibility of recruitment, safety and acceptability of the hyperoxygenation test in pregnant women.
2. To evaluate the fetal response to maternal hyperoxygenation in singleton pregnancies in the third trimester.
3. To evaluate if pulmonary artery reactivity to maternal hyperoxygenation identifies fetuses that will develop pulmonary hypertension in the early newborn period.

Secondary objectives

1. To correlate neonatal echocardiographic findings with fetal echocardiographic changes in response to maternal hyperoxygenation.
2. To assess serial changes in maternal cardiac output, stroke volume and systemic vascular resistance before, during and after maternal hyperoxygenation.

1.14 Pilot Study

"Developing, piloting, evaluating, reporting and implementing a complex intervention can be a lengthy process. All of the stages are important, and too strong a focus on the main evaluation, to the neglect of adequate development and piloting work, or proper consideration of the practical issues of implementation, will result in weaker interventions, that are harder to evaluate, less likely to be implemented and less likely to be worth implementing."

The above quotation from the Medical Research Council framework (287) is pertinent to this work. This thesis represents preliminary work, an extremely important step prior to the organisation of a much larger-scale trial. Many funding streams, such as the UK National Institute for Health Research (NIHR), Research for Patient Benefit (RfPB) (288) and the United States NIH Planning Grant Program-R34 (289) funding mechanism now acknowledge the importance of preliminary work through the provision of substantial monetary supports for many such projects. There is now increased recognition of the value of undertaking preliminary work and researchers are encouraged to publish their pilot data (290). A pilot study is a requisite and an essential initial step in examining a novel intervention or an innovative application of an intervention in advance of a main trial. The terms 'feasibility' and 'pilot' study are used interchangeably in the literature and the distinction between the two is not clear cut (290-292). However, it has been reported

that the main focus of a pilot study should be to test the feasibility of conducting a full scale study rather than the statistical significance of the gathered data (293). What is clear is that pilot studies can be used to evaluate the feasibility of recruitment, randomisation, retention, assessment procedures, new methods and implementation of the novel intervention (289). Therefore, pilot studies can inform feasibility and aid in identifying any modifications needed in the design of a larger trial. The main aim of a pilot study is to assess the potential for successful implementation of the proposed intervention and to reduce threats to the validity of these studies (294). A review of the literature performed by Whitehead et al (290) summarised that the distinguishing features of a pilot study from a feasibility study were as follows:

- Stricter study methodology (e.g. a justification of the sample size)
- An intention for further work
- Smaller version of the main study (e.g. use of a control group and randomisation)
- A focus on trial processes

All of the above features can be applied to our study.

2 CHAPTER 2 Materials and Methods

2.1 The HOTPOT Study

The HOTPOT study (Can the **H**yper**O**xygenation **T**est **P**redict neonatal pulm**O**nary hyper**T**ension?) was conducted in the Rotunda Hospital, Dublin, Ireland between January 2017, and July 2018. This pilot study was designed to assess the feasibility of recruitment, safety and acceptability of the hyperoxygenation test in pregnant women. This study also aimed to identify if MH in pregnancy could predict neonatal pulmonary hypertension.

This was a multidisciplinary project with input from the obstetric, neonatal and perinatal anaesthesia departments at the Rotunda Hospital. The study was funded by the Rotunda Foundation (formerly known as the Friends of the Rotunda). The Rotunda Foundation organisation was established in 1971 primarily to raise funds for Rotunda-based research and this project is an excellent example of a collaborative research project, based at the hospital. This study was carried out solely in the Rotunda Hospital, a tertiary referral maternity centre with over 8,500 deliveries per annum. The neonatology department at the hospital accept national referrals and have over 1,500 admissions to the neonatal unit per year. The hospital also has a neonatal intensive care unit (NICU) and an outpatient department on site, which allowed for follow up of the postnatal paediatric outcomes.

The study enrolled eligible patients to undergo a comprehensive fetal ultrasound scan and echocardiogram and to undergo the maternal hyperoxygenation test for a duration of ten minutes. This was followed by a repeat fetal ultrasound scan and echocardiogram. Serial assessments of maternal haemodynamics using bio-reactance technology with NICOM® (Cheetah Medical, Maidenhead, Berkshire, United Kingdom) were acquired over the duration of the study period. Neonatal echocardiography was performed on the infants of the mothers who received the hyperoxygenation test. The NICOM®-acquired haemodynamics, maternal variables, pregnancy outcomes, delivery outcomes, neonatal characteristics and neonatal echocardiographic indices were recorded for all study participants and maintained on a centralised database on an encrypted laptop and encrypted external hard drive for data management and analysis.

This PhD project is novel. To undertake this work, I trained in the area of fetal cardiology for three years under the guidance and supervision of Dr Orla Franklin and Professor Fionnuala Breathnach. I began training in fetal ultrasound in July 2016 under the supervision of Professor Breathnach. I gained competence in performing and measuring fetal biometry, amniotic fluid indices and in obtaining routine fetal Doppler measurements including those of the UA, MCA, and DV. I attended a minimum of three to five fetal medicine scan lists per week over a three-year period to consistently practise and to gain competence in both fetal ultrasound and fetal echocardiography. To gain proficiency in obtaining detailed fetal 98

echocardiography images, including pulmonary artery, aortic isthmus and ductus arteriosus Doppler waveform acquisition, I attended a weekly fetal echocardiography scan list with Professor Breathnach and a monthly fetal echocardiography scan list with both Professor Breathnach and Dr Franklin. I became skilled in performing detailed fetal echocardiography over three years and this allowed me to have confidence in the measurements and images that I obtained at each patient encounter over the study period. I applied for a EudraCT number, developed the study protocol, created, and submitted a health products and regulatory authority (HPRA) protocol and obtained approval. I was successful in obtaining funding for the study through a competitive application process. I applied for and obtained ethical approval to undertake the study. I worked closely with our data safety monitor from the creation of the study concept, development of study protocols, case report files, consent forms, patient information leaflets, through to the site initiation visit and regular data safety monitoring meetings to the close out visit of the study. I was actively involved in all aspects of this project. I performed all the fetal and maternal assessments. I recruited and consented all patients. I performed all fetal ultrasound scans and fetal echocardiograms. I administered all hyperoxygenation tests and attached all NICOM® monitors. I retrieved all maternal and fetal data and undertook all postnatal follow-up assessments. I was consistently supported through the study by my three supervisors.

2.2 Study Timeline

Activity	**Date approved/accepted**
Registered PhD RCSI April 2016	May 2016
Protocol Development	Began July 2016-Complete January 2017
Funding application July 2016	Approval received September 2016
Application for EudraCT number July 2016	Accepted July 2016
Application to HPRA August 2016	Approval from HPRA October 2016
Application for national ethical approval. August 2016	Approval by Ethics committee September 2016
Application for Sponsorship August 2016	Sponsorship approval from RCSI September 2016
Sponsor Feasibility study visit October 2016	Completed October 2016
Site Initiation Visit (SIV)	Completed November 2016
Issue of study contract from Funder November 2016	Received November 2016
Study Contract and draft Clinical Trial agreement (CTA) submitted November 2016	CTA January/February 2017
Case report forms, patient information leaflets, consent forms finalised	January 2017
First patient recruitment	February 2017

2.3 Study Design and Methods

2.3.1 Study Design

This was a prospective pilot study of 66 women (46 pregnant and 20 non-pregnant). On recruitment to the study, participants completed a written consent form, were assigned a study number and had basic demographics and vital signs assessed. Pregnant participants underwent a detailed fetal third trimester ultrasound scan and fetal echocardiogram. The maternal hyperoxygenation test was performed, and the fetal ultrasound measurements were repeated. The NICOM® assessment commenced ten minutes prior to the administration of oxygen and continued for at least ten minutes following the cessation of hyperoxygenation. Non-pregnant women were recruited as a comparison group. Non-pregnant subjects were recruited through the Gynaecology department and included research and clinical staff members who were interested in enrolling in the study. Controls were matched for age and body mass index (BMI) (relating to the pregnant patients booking BMI) to allow for comparison. The non-pregnant subjects underwent the same procedure in relation to hyperoxygenation and NICOM® assessment, however they did not have any ultrasound procedure performed. Once the pregnant participants had delivered their baby, one of the neonatal study investigators was informed and a comprehensive neonatal echocardiogram was performed. The mothers were followed up at six weeks postnatal either in the hospital or by telephone. The HOTPOT

study was observational and descriptive in nature, there were no pre-defined management or delivery criteria and all clinical decisions were made by the lead clinician managing the case, who was blinded to the fetal and maternal responses to MH.

The fetal response was divided into 2 cohorts

1. Those who responded to maternal hyperoxygenation

   (Defined as a decrease in the PA PI of ≥10% from the baseline as previously defined (110).

2. Those who did not respond to maternal hyperoxygenation

   (Defined as a decrease in the PA PI of <10% from the baseline).

Defining PPHN

Neonatal persistent pulmonary hypertension was defined by neonatal echocardiography as well as by clinical indicators as follows:

1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,

2) Normal structural anatomy of the heart on echocardiogram (with the exception of those recruited to Group C of the study with a known ventricular septal defect (VSD) or atrioventricular septal defect (AVSD)) and,

3) In the presence of a tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) of ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or

4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic)

5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity.

2.3.2 Clinical Data and Outcome Measures

Maternal data

The following maternal details were collected at enrolment by patient interview and a chart review:

- Maternal age
- Maternal weight (kg), height (cm) and BMI (kg/m^2)
- Ethnicity (Classed as Caucasian, Indian/Pakistani/Bangledesi, Afro-Caribbean, African (Sub-Saharan), Middle-East/North Africa, Asian or Other)
- Smoking status
- Medical history

- Medication use (including current medications or medication use within the last seven days)
- Obstetric history
- Most recent Hb level (g/dL)

Maternal records were matched to neonatal charts and demographic and outcome data were abstracted. Neonatal records provided information on:

- Gestational age at delivery (weeks)
- Birthweight (grams)
- NICU course

2.3.2.1 Primary Objectives

The primary study objectives were:

1. To assess feasibility of recruitment, safety and acceptability of the hyperoxygenation test in pregnant women.

2. To evaluate the fetal response to maternal hyperoxygenation in singleton pregnancies in the third trimester.

3. To evaluate if pulmonary artery reactivity to maternal hyperoxygenation identifies fetuses that will develop pulmonary hypertension in the early newborn period.

The secondary study objectives were:

1. To correlate neonatal echocardiographic findings with fetal echocardiographic changes in response to maternal hyperoxygenation.

2. To assess serial changes in maternal cardiac output, stroke volume and systemic vascular resistance before, during and after maternal hyperoxygenation.

An assessment of the feasibility, acceptability, and safety of the maternal hyperoxygenation test in pregnancy was measured by the following:

2.3.2.2 Feasibility

- The proportion of eligible women that were approached who agreed to participate in the study
- The proportion of women in whom it was possible to obtain PA Doppler velocimetry measurements
- Satisfactory collection of all endpoints and variables
- Specific study protocol violations

2.3.2.3 Acceptability

- Adherence to study procedures (Fetal ultrasound, NICOM® monitoring and Oxygen administration)
- Attendance at study visits
- Questionnaire following MH comprising of three questions:

- 1. Was the oxygen test comfortable?
- 2. Was the overall test too long?
- 3. Would you undergo this test again?

2.3.2.4 Safety

- Monitoring of maternal haemodynamics

2.3.3 Eligibility Criteria and Recruitment

2.3.4 Patient Population

Pregnant women who had attained a minimum GA of 31 weeks and up to 40 weeks were recruited to the study. The patients were recruited through the prenatal department in the hospital. If deemed eligible, I approached patients attending the ultrasound department or current inpatients and asked them if they would like to partake in the study. Only patients with adequate knowledge of the English language were invited to participate. All patients recruited to the study were provided with a patient information leaflet, assigned a study number and provided written informed consent prior to participation. I collected all baseline demographics as detailed above. Any medical problems and current medications, initial systolic and diastolic BPs and GA were recorded. Gestational age was confirmed by an early dating ultrasound report.

2.3.5 Inclusion Criteria

Inclusion criteria included five separate subgroups:

A) Women carrying a fetus at risk of pulmonary hypoplasia: (for example mid-trimester PPROM, CDH, skeletal dysplasia, persistent oligohydramnios)

B) Women attending for scheduled CS prior to 38 weeks' GA

C) Women with a prenatal diagnosis of moderate/severe perimembranous VSD or AVSD in the fetus in the absence of other structural heart disease, including fetuses with Trisomy 21

D) A group of gestation-matched uncomplicated singleton pregnancies to serve as a control group

E) Non-pregnant controls

In addition, the following inclusion criteria were applied:

- Singleton pregnancies with a normally grown fetus (estimated fetal weight (EFW) ≥5th centile and ≤95th centile for GA)
- ≥31 weeks and ≤40 weeks gestation
- Subjects that were able and willing to give written informed consent and to comply with the requirements of the study protocol

- Female subjects, aged 18 years or above at baseline
- Subjects judged to be in generally good health by the investigator based upon the results of the medical history
- Subjects with a non-smoking status

2.3.6 Exclusion Criteria

Exclusion criteria were as follows:

- Age <18 years
- Known diagnosis of non-Down aneuploidy
- Gestational age <31 weeks and >40 weeks
- Maternal chronic respiratory disease (including chronic obstructive pulmonary disease, cystic fibrosis and pulmonary fibrosis)
- Maternal congenital heart disease (CHD)
- Maternal use of bleomycin or amiodarone (in animal models, the toxic effects of hyperoxia and bleomycin are synergistic, resulting in more extensive lung injury and fibrosis (295)
- Subjects unable to provide written informed consent
- Subjects with any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to

severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, put the subject at risk by participation in the study, or may influence the result of the study

- Smokers

2.3.7 Justification for Inclusion and Exclusion Criteria

Only normally grown fetuses were included with an EFW ≥5th centile and ≤95th centile for GA to exclude any effect that growth restriction (257, 296, 297) or fetal macrosomia (298, 299) may have on fetal Doppler waveforms. Fetuses' ≥31 weeks' gestation were included as the hyperoxygenation test is known to become responsive after this GA (54). Those with a GA ≥40 weeks were excluded given the potential for advanced GA to affect the acquisition of or the result of various Doppler indices. A window of 31-40 weeks GA was chosen to increase uniformity and to acquire better data. Participants with a non-smoking status were chosen given the hazards associated with smoking and high flow oxygen (300) and to eliminate any effect that smoking may have on Doppler velocity waveforms (301-303). Although many women with CHD can go through pregnancy with a low risk to themselves, there remains a higher incidence of miscarriage, premature births, low birth weights and an increase of CHD in the fetus of women with cyanotic CHD than what is found in the normal population (304). Notwithstanding that there is an increased

risk of cardiac and neonatal complications associated with maternal CHD in pregnancy (305), there is limited data on the haemodynamic changes in pregnancy in response to MH and for this reason we excluded both women with CHD and those with any chronic respiratory disease.

2.3.8 Study Groups

Group A

This group was comprised of women who were carrying a fetus at risk of pulmonary hypoplasia (including mid-trimester PPROM, CDH, skeletal dysplasia, and persistent oligohydramnios). Within this group, PPROM was defined as rupture of the fetal membranes before 37 weeks gestation and more than 24 hours prior to the onset of labour. Persistent oligohydramnios was defined as a single deepest pocket (SDP) of amniotic fluid of less than 2cm and a duration of seven days between the diagnosis of oligohydramnios and recruitment to the study. The fetal conditions within this group all carry an increased risk of PPHN, these include mid-trimester PPROM (306), CDH (307), skeletal dysplasia (308) and persistent oligohydramnios (309, 310).

Group B

This group was comprised of women attending for scheduled CS prior to 38 weeks' GA. This group was included given the increased risk of PPHN in this cohort (311-313).

Group C

This group comprised of women with a prenatal diagnosis of a moderate or severe perimembranous VSD or AVSD in the fetus in the absence of other structural heart disease, including fetuses with Trisomy 21. This group was included given the increased risk of respiratory morbidities and PPHN in these neonates (314, 315).

Group D

This group was comprised of pregnant women in the third trimester ≥31 weeks GA with no known fetal abnormality or prenatal risk factor for PPHN. The purpose of this group was to serve as gestation-matched uncomplicated controls. The findings in this group were going to be useful when comparing the reactivity of normal pregnancies with those at risk for PPHN. This group was likely to give us further insights into the feasibility and acceptability of the MH test.

Group E

This group was comprised of non-pregnant women. The main purpose of the non-pregnant controls was to serve as a comparator group when analysing the haemodynamic changes that occur in response to MH.

2.3.9 Period of Study

This prospective single centre pilot study was carried out at the Rotunda Hospital, Dublin, Ireland. The Rotunda hospital is a stand-alone tertiary referral maternity hospital with over 8,500 deliveries annually. The recruitment phase of the study ran from January 2017 to July 2018. All patients completed the study protocol including a six-week postnatal review by September 2018.

2.3.10 Statistical Analysis

Descriptive statistics were used to summarise the findings into two groups, responders and non-responders. Normally distributed data are reported as means and standard deviations (SD) while non-normally distributed data are reported as medians and interquartile ranges (IQR). Maternal and neonatal characteristics were compared using Chi-square or Fisher’s exact test for frequencies, or Student's t-test or Wilcoxon rank-sum test (Mann-Whitney U test), for normally distributed or non-normally distributed continuous

variables, respectively. Correlations between the neonatal echocardiographic findings and the response to MH were assessed using Pearson's correlation for normally distributed data, Spearman's correlation for non-normally distributed data. All tests were two-tailed and the significance level for all analyses was set at $p < 0.05$. Statistical analysis was performed using SPSS (version 24.0). This study was conducted in accordance with the STROBE guidelines (316).

2.3.10.1 Analysing Haemodynamics

Data were tested for normality using the Shapiro-Wilk test and a histogram representation of data. Continuous variables were presented as means ± SD or medians [IQR] as appropriate. Two group comparisons were performed using the Student t-test or the Mann Whitney U test as appropriate. Two-way ANOVA with repeated measures was used to assess the change in the haemodynamic measurements over time and between the two groups (pregnant and non-pregnant). Pairwise comparisons were performed to assess the difference between timepoints 1 and 2 and timepoints 1 and 3 (Timepoints explained further in *section 2.4.2*). A post-hoc power calculation to judge the appropriateness of our sample size was performed (based on the lower number of 20 subjects in the non-pregnant group). Power analysis based on a total peripheral resistance (TPR) or SVR difference of 300 dynes/sec/cm^{-5} between the groups with a SD of 350dynes/sec/cm^{-5} provides

a power of 0.80 and an error probability associated with this test of this null hypothesis of 0.05.

2.3.10.2 Analysing Repeatability

In order to assess the reproducibility of the measured PA parameters, fetal echocardiograms and Doppler velocity waveform measurements from the PA of 10 fetuses that were not included in the study were recorded. Intraobserver and interobserver variability of PA indices were assessed using this subset of 10 fetuses. One reader (A.M) repeated measurements at a time temporally remote from the initial assessment (approximately 15 minutes). To assess interobserver variability, a second reader (F.B), blinded to the original data, repeated PA measurements. Intraobserver and interobserver variability was assessed using the intraclass correlation coefficient (ICC). To assess the repeatability of the measured values, the mean and SD of differences and the repeatability coefficient of the two repeated tests within subjects were calculated (317). To estimate the SD of the differences between the two repeated tests, it was assumed that the true mean difference was equal to zero. The repeatability coefficient has been defined as 1.96 times the SD of differences between repeated measurements (223, 317). Additionally, the coefficient of variation (CV) was calculated from the two repeated tests. The CV was defined as the SD of the error in a single test and expressed as a percentage of the mean PA PI (223).

2.3.10.3 Determination of Sample Size

Sample sizes of between 24 (12 per group) and 50 have been recommended variously for pilot studies (288, 318-324). Following these recommendations, we chose a recruitment sample size of 60-75 (12 subjects per group with a potential addition of 20 subjects in group E) which would allow for a moderate dropout rate. A significant dropout rate (e.g. 40%) would reduce the pilot sample size to below a minimum of 24, in which case a planned larger study would be called into question in the first place, having possible external validity issues, pragmatic or ethical concerns. Fifteen to 30 subjects were planned to be in the normal pregnancy control group. Whether from group A, B, C or D, we hypothesised that some subjects would have a reactive hyperoxygenation test and others would have a non-reactive hyperoxygenation test. Therefore, there would be two groups (responders and non-responders).

Dr. Patrick Dicker, a medical biostatistician at the RCSI Department of Obstetrics and Gynaecology, and Department of Epidemiology and Public Health performed the initial power calculation for the study. Prof Afif- El Khuffash and I performed all data checks and statistical analyses for the study.

2.4 Overview of the Study Procedures

2.4.1 Measurement of the Maternal Haemodynamics

The NICOM® machine was used to assess the haemodynamic profile of the pregnant and non-pregnant participants. I was trained in using the machine prior to the initiation of the study. Training included a step-by-step instruction on the application of the equipment, in how to navigate the machine controls, obtain the haemodynamic data and in how to archive the data for later analysis. Prior to the commencement of the study, a training session was set up and led by Ms Lisa McSweeney, a research assistant working in the Rotunda Hospital who had previously attended a training day led by a representative from Cheetah Medical. Further technical assistance was available from Cheetah Medical.

The NICOM® equipment consists of the NICOM® machine and four single use electrodes *(Figure 2.1).* The monitor was turned on to allow for haemodynamic monitoring. The participant's age, gender, height, current weight, and HOTPOT study identification number were inputted onto the NICOM® device. Haemodynamic monitoring was performed with the participant lying in a semi-recumbent position to avoid aortocaval compression (in the pregnant cohort). Four emitting and receiving NICOM® electrodes were attached to the participant's chest. Two upper thoracic electrode strips were placed over the mid-clavicles in the mid clavicular line bilaterally and two lower electrode sensors were placed at the costal margin

in the mid-clavicular line *(Figure 2.2).* They were placed on the chest in this manner to “box” the heart. Brachial artery BP measurements were recorded at 5-minute intervals using a standard BP cuff that was placed on the participant’s upper left arm. The NICOM® was allowed to calibrate and then haemodynamic monitoring continued for the duration of all study procedures. The HOTPOT study was observational and descriptive in nature and therefore there were no pre-specified cut off limits for haemodynamic variables.

Figure 2.1 NICOM® Machine and Single Use Electrodes

Image of the NICOM® machine and the single use electrodes.

(Image courtesy of Cheetah Medical).

Figure 2.2 Application of NICOM® Electrodes

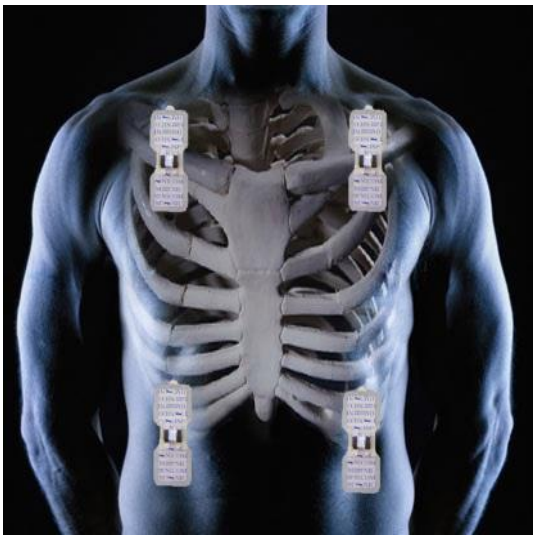


Illustration of the placement of the NICOM® electrodes in a manner that " boxes" the heart. Two electrodes were placed on the upper thorax and two electrodes were placed on the lower thorax in the mid-clavicular line.

(Image courtesy of Cheetah Medical).

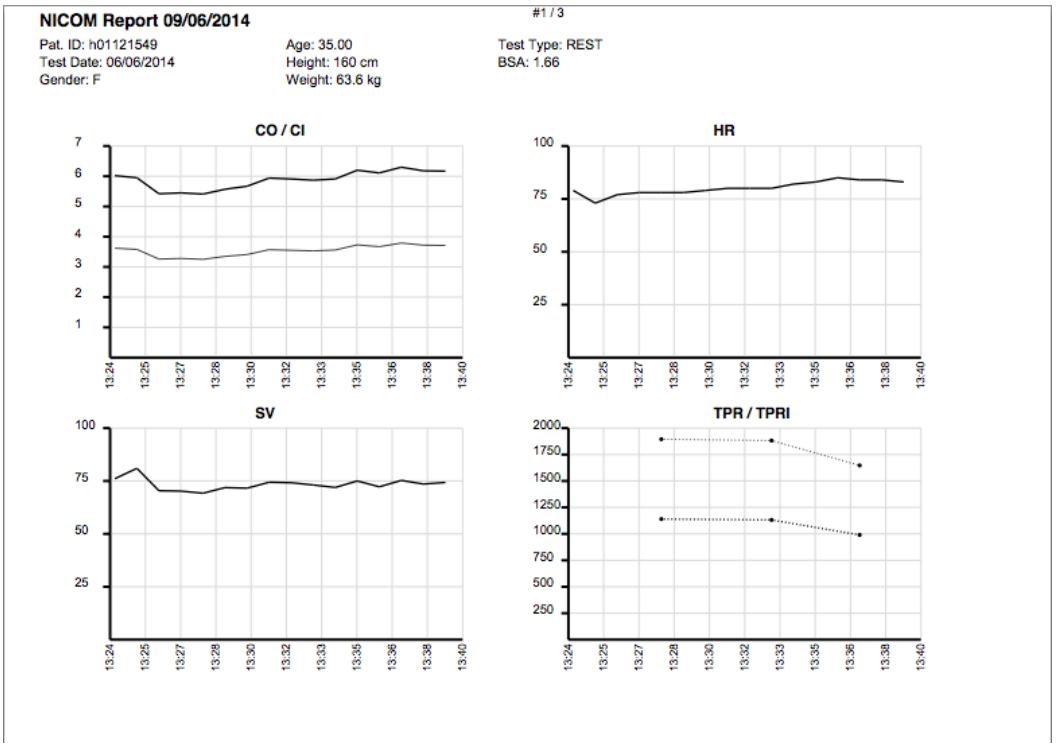
2.4.2 Retrieval of the NICOM® Data

To retrieve the NICOM® data, the device was powered on and connected to the study laptop. Device mode was selected by scrolling to the settings tab and pressing on the menu icon. Once in device mode, the data transfer could occur. On the study desktop computer, NICOM® electronic medical records (EMR) (version 1.5.5) was selected. The retrieve data icon was selected from the drop-down menu and the on-screen instructions were followed. This enabled the data to be exported to the laptop. The data report was viewed as either a digital diagram *(Figure 2.3 and 2.4)* or as a three-page PDF executive report as detailed in *Figure 2.5*. This report was saved to the study laptop. Values corresponding to the study times were recorded and inserted onto an excel spreadsheet *(Figure 2.6).* Three time points were assessed as follows:

- Time point 1- Baseline (Pre MH)
- Time point 2- At ten minutes of MH
- Time point 3- Ten minutes following the cessation of MH

The values were then inserted under the selected patient and timepoint in the study database. For a subset of 22 pregnant participants a Timepoint 4 was recorded. Timepoint 4 was at 30 minutes following the cessation of MH.

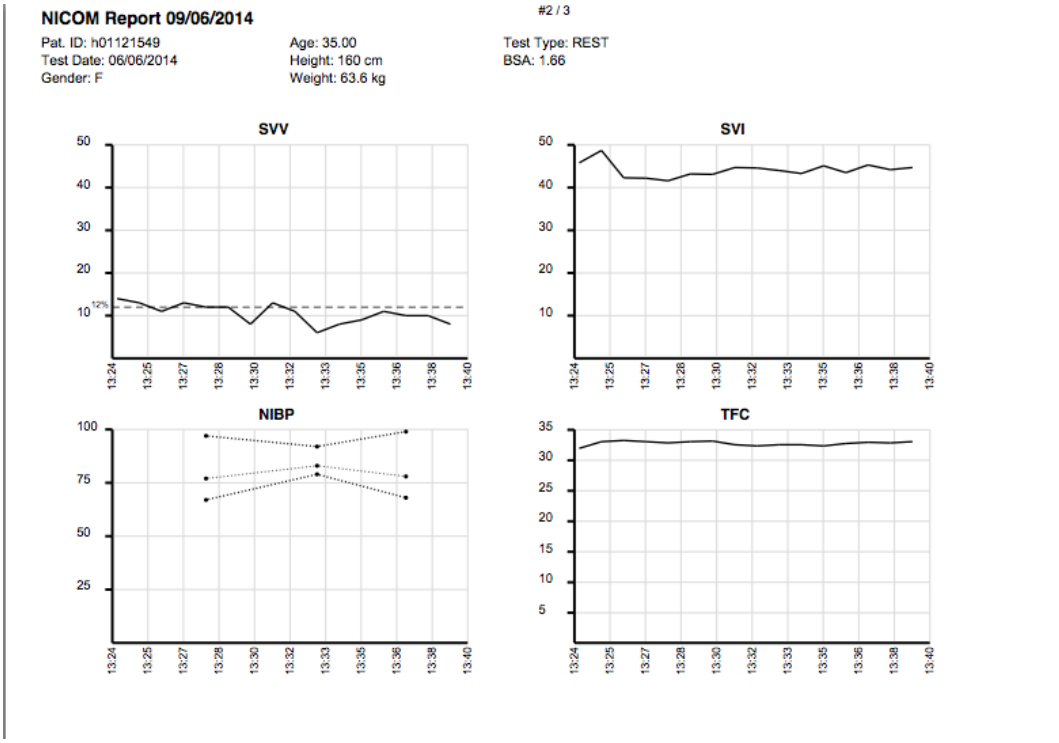
Figure 2.3 NICOM® Example Report Page 1



Abbreviations: CO, cardiac output; CI, cardiac index; HR, heart rate; SV, stroke volume; TPR, total peripheral resistance; TPRI, total peripheral resistance indexed; Pat. ID, patient identification; F, female; BSA, Body surface area.

(Image courtesy of Cheetah Medical).

Figure 2.4 NICOM® Example Report Page 2



Abbreviations: SVV; stroke volume variation; SVI, stroke volume indexed; NIBP, non-invasive blood pressure; TFC, thoracic fluid content, F- female; BSA- body surface area; Pat. ID, patient identification.

(Image courtesy of Cheetah Medical).

Figure 2.5 NICOM® Example Report Page 3

Report Date: 09/06/2014

Pat. ID: h01121549

Test Date: 06/06/2014

#3 / 3

Samples

Sample #	S.Time	CO	CI	HR	NIBP	MAP	TPR	TPRI	SV	SVI	SVV	SPO2	DO2I	TFC
1	13:24:13	6	3.6	79	--/--	--	--	--	76.1	46	14%	--	--	31.9
2	13:25:13	5.9	3.6	73	--/--	--	--	--	81	49	13%	--	--	33
3	13:26:13	5.4	3.3	77	--/--	--	--	--	70.4	42	11%	--	--	33.2
4	13:27:13	5.4	3.3	78	--/--	--	--	--	70.2	42	13%	--	--	33
5	13:28:13	5.4	3.2	78	97 / 67	77	1140	1895	69.2	42	12%	--	--	32.8
6	13:29:13	5.6	3.3	78	--/--	--	--	--	71.9	43	12%	--	--	33
7	13:30:13	5.7	3.4	79	--/--	--	--	--	71.6	43	8%	--	--	33.1
8	13:31:13	5.9	3.6	80	--/--	--	--	--	74.4	45	13%	--	--	32.5
9	13:32:13	5.9	3.5	80	--/--	--	--	--	74.2	45	11%	--	--	32.3
10	13:33:13	5.9	3.5	80	92 / 79	83	1132	1882	73.1	44	6%	--	--	32.5
11	13:34:13	5.9	3.6	82	--/--	--	--	--	71.9	43	8%	--	--	32.5
12	13:35:13	6.2	3.7	83	--/--	--	--	--	75	45	9%	--	--	32.3
13	13:36:13	6.1	3.7	85	--/--	--	--	--	72.3	44	11%	--	--	32.7
14	13:37:13	6.3	3.8	84	99 / 68	78	990	1647	75.3	45	10%	--	--	32.9
15	13:38:13	6.2	3.7	84	--/--	--	--	--	73.6	44	10%	--	--	32.8
16	13:39:13	6.2	3.7	83	--/--	--	--	--	74.3	45	8%	--	--	33

Abbreviations: CO, cardiac output; CI, cardiac index; HR, heart rate; NIBP, non-invasive blood pressure; MAP, mean arterial pressure; TPR, total peripheral resistance; TPRI, total peripheral resistance indexed; SV, stroke volume; SVI; stroke volume indexed; SVV, stroke volume variation; SPO_2, Arterial Hemoglobin Oxygen Saturation; DO_2I, Oxygen Delivery Index; TFC, thoracic fluid content.

(Image courtesy of Cheetah Medical).

Figure 2.6 NICOM® Example Excel Report

	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	'Study No'	'CO'	'CI'	HR	NIBP	'MAP'	'manMAP'	'TPR'	'TPRI'	'CP'	'CPI'	'SV'	'SVI'
2	1	6.02	3.62	79	--/--							76.09	46
3	2	5.95	3.58	73	--/--							80.97	49
4	3	5.42	3.26	77	--/--							70.39	42
5	4	5.45	3.28	78	--/--							70.21	42
6	5	5.41	3.25	78	97/67	77		1140	1895	0.9	0.6	69.22	42
7	6	5.57	3.35	78	--/--							71.87	43
8	7	5.67	3.41	79	--/--							71.6	43
9	8	5.94	3.57	80	--/--							74.4	45
10	9	5.91	3.55	80	--/--							74.18	45
11	10	5.87	3.53	80	92/79	83		1132	1882	1.1	0.6	73.12	44
12	11	5.91	3.56	82	--/--							71.94	43
13	12	6.2	3.73	83	--/--							75.01	45
14	13	6.11	3.67	85	--/--							72.26	44
15	14	6.3	3.79	84	99/68	78		990	1647	1.1	0.7	75.27	45
16	15	6.18	3.72	84	--/--							73.56	44
17	16	6.17	3.71	83	--/--							74.3	45

Abbreviations: CO, cardiac output; CI, cardiac index; HR, heart rate; NIBP, non-invasive blood pressure; MAP, mean arterial pressure, TPR, total peripheral resistance; TPRI, total peripheral resistance indexed; CP, cardiac power; CPI, cardiac power index; SV, stroke volume, SVI, stroke volume indexed.

(Image courtesy of Cheetah Medical).

2.4.3 Fetal Ultrasound

All ultrasound examinations were performed using a Voluson E8 Expert ultrasound system (GE Healthcare, Milwaukee, WI, USA) equipped with a 4–6-MHz convex transducer (RAB-6 Wide Band Convex Volume Transducer, Curved Array Volume Transducer). All pregnant participants underwent a standard ultrasound examination for EFW, amniotic fluid volume and fetal HR. All pregnant participants were scanned in a semi-recumbent position at a 45-degree angle slightly tilted to the left side to avoid aortocaval compression. Study participants were not made aware of their response to the hyperoxygenation test at the time of the ultrasound.

2.4.4 Fetal Biometry assessment

Gestational age was calculated from the last menstrual period according to Naegele's rule (325) and confirmed by a first trimester ultrasound dating scan between 8-14 weeks gestation, based on a fetal crown rump length (CRL). In the presence of disparities between the two methods, the GA was calculated from the sonographic measurement of the fetal CRL (326).

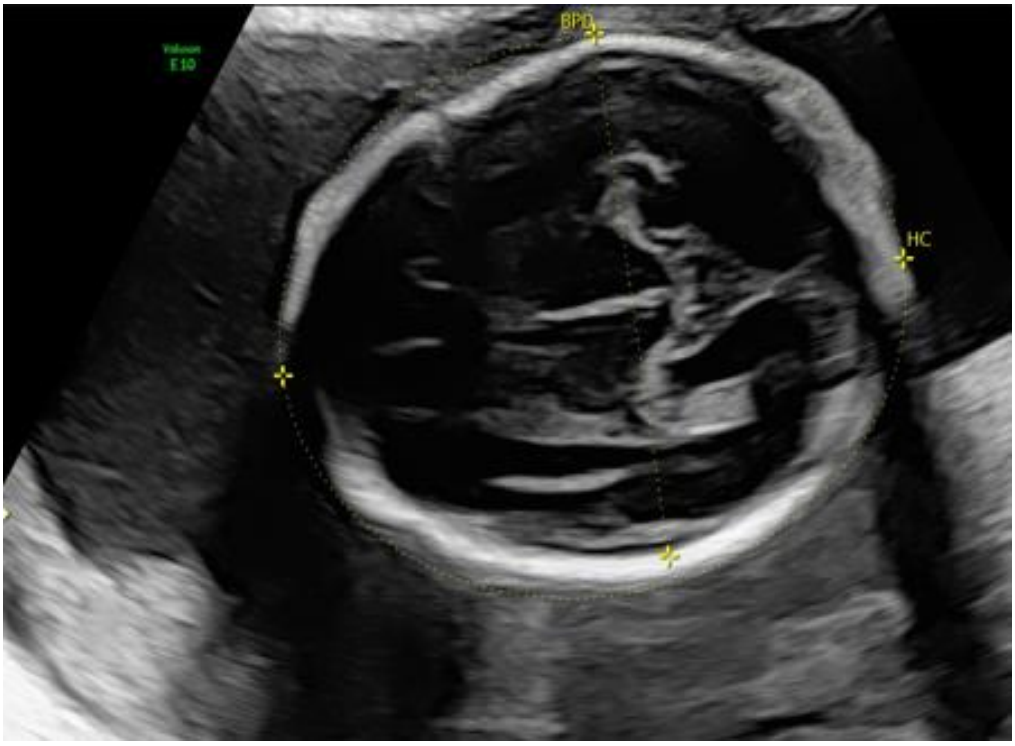
Fetal biometry provided an EFW in grams (g). It was calculated by combining the measurements of the fetal head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL). The fetal head was examined in an axial plane with the thalami and cavum septum pellucidum visualised. The ultrasound transducer remained

perpendicular to the central axis of the fetal head with the hemispheres and calvaria appearing symmetric. To measure the BPD, the calipers were placed at the outer edge of the near calvarial wall and then at the inner edge of the far calvarial wall (outer to inner). The fetal HC was measured in the same image using an ellipse, which was drawn around the outside of the calvarium (327) *(Figure 2.7).* The fetal AC was measured in a transverse plane of the fetal abdomen with the abdominal segment of the UV seen at the level of the portal sinus. The stomach bubble and the outer surface of the skin line was included in the area measured (328). The fetal AC was measured using the ellipse facility of the machine by placing the calipers on the outer edges of the circumference *(Figure 2.8).* The fetal FL was measured in the longitudinal plane and included the proximal and distal femoral diaphysis excluding the proximal and distal epiphysis *(Figure 2.9)* (329). The EFW was calculated automatically using the Hadlock 4 equation:

Log10EFW= 1.3596- 0.00386(AC x FL) + 0.0064(HC) + 0.00061 (BPD x AC) + 0.0425(AC) + 0.174 (FL) (304).

Amniotic fluid indices were based on a single deepest vertical pool measurement (DVP) or single deepest pocket (SDP) (330), which included the greatest vertical dimension of the deepest pocket of amniotic fluid free of umbilical cord and fetal extremities.

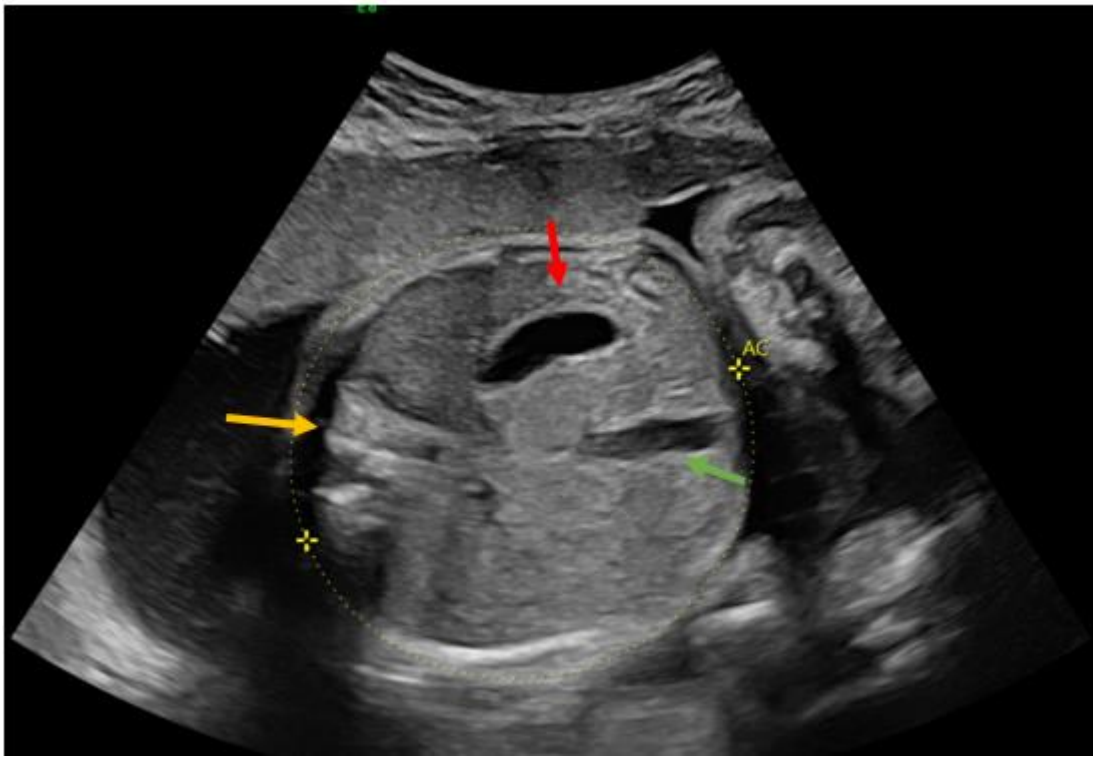
Figure 2.7 Ultrasound Measurement of Fetal Biparietal Diameter and Head Circumference



Ultrasound image of a fetal biparietal diameter and head circumference measurement.

Abbreviations: BPD, biparietal diameter; HC, head circumference.

Figure 2.8 Ultrasound Measurement of Fetal Abdominal Circumference

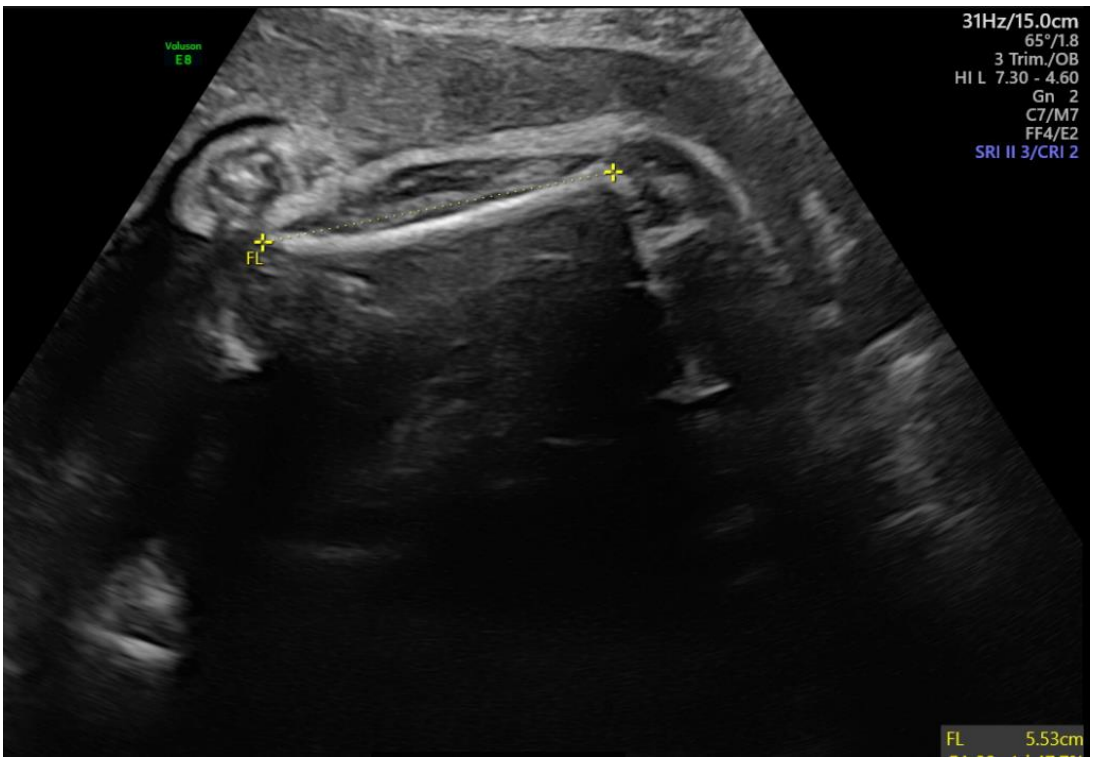


Ultrasound image of a fetal abdominal circumference measurement.

Abbreviations: AC, abdominal circumference.

Red arrow marks the stomach; Green arrow marks the umbilical vein; Yellow arrow marks the vertebral body.

Figure 2.9 Ultrasound Measurement of Fetal Femur Length



Ultrasound image of a fetal femur length measurement.

Abbreviations: FL, femur diaphysis length.

2.4.5 Doppler Sonography Standards

Image-directed pulsed and colour Doppler equipment (Voluson E8, GE Healthcare) was used with a transabdominal 5-MHz sector probe to obtain blood velocity waveforms. The lowest high-pass filter level (100Hz) was used and the spatial peak temporal average power output for colour and pulsed Doppler was kept at <100mW/cm. The high-pass filter was employed to remove the wall component from the blood flow signal (331). The spatial peak temporal average power output is the maximum intensity occurring in an ultrasound beam averaged over the pulse repetition period. For pulsed ultrasound assessment this is the time from the beginning of one pulse to the beginning of the next.

2.4.6 Factors Affecting Doppler Acquisition

Fetal HR and the length of the fetal cardiac cycle have an inverse relationship. Therefore, fetal HR can influence the configuration of the ultrasound derived arterial Doppler waveforms (332). If the fetal HR is within normal limits (110bpm-160bpm) (333), the change in arterial Doppler waveform configuration bears no clinical significance. However, if the fetal HR drops, the diastolic phase of the cardiac cycle is prolonged, and the end-diastolic frequency shift will decline. In addition, fetal breathing movements can have an effect on the shape of the Doppler waveform and examinations should be conducted only during fetal apnea and in the absence of

excessive fetal movements (334). All Doppler measurements were taken during periods of fetal quiescence and while all fetuses were in sinus rhythm.

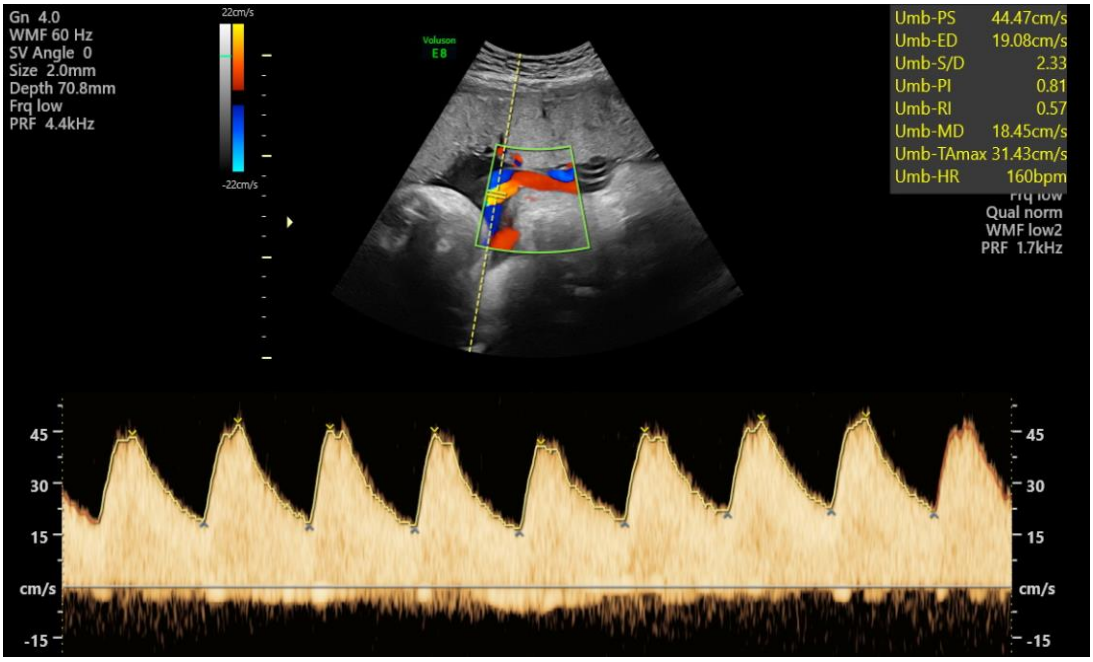
2.4.7 Umbilical Artery Doppler

The UAD waveforms were obtained by identifying a free loop of umbilical cord. The Doppler sample gate was then placed over the umbilical artery. The waveform indices were measured using the automatic function on the ultrasound machine *(Figure 2.10).*

2.4.8 Middle Cerebral Artery Doppler

The MCA Doppler was obtained in an axial section of the fetal brain. This includes the thalami and the wings of the sphenoid bone. The Circle of Willis and the proximal MCA were identified using colour Doppler mapping. Once identified, the pulsed wave sample gate was placed over the proximal third of the MCA. The angle of insonation was kept as close to 0° as possible *(Figure 2.11).* The cerebro-placental (CPR) was calculated as the ratio of the MCA PI to the UA PI, as previously described (335).

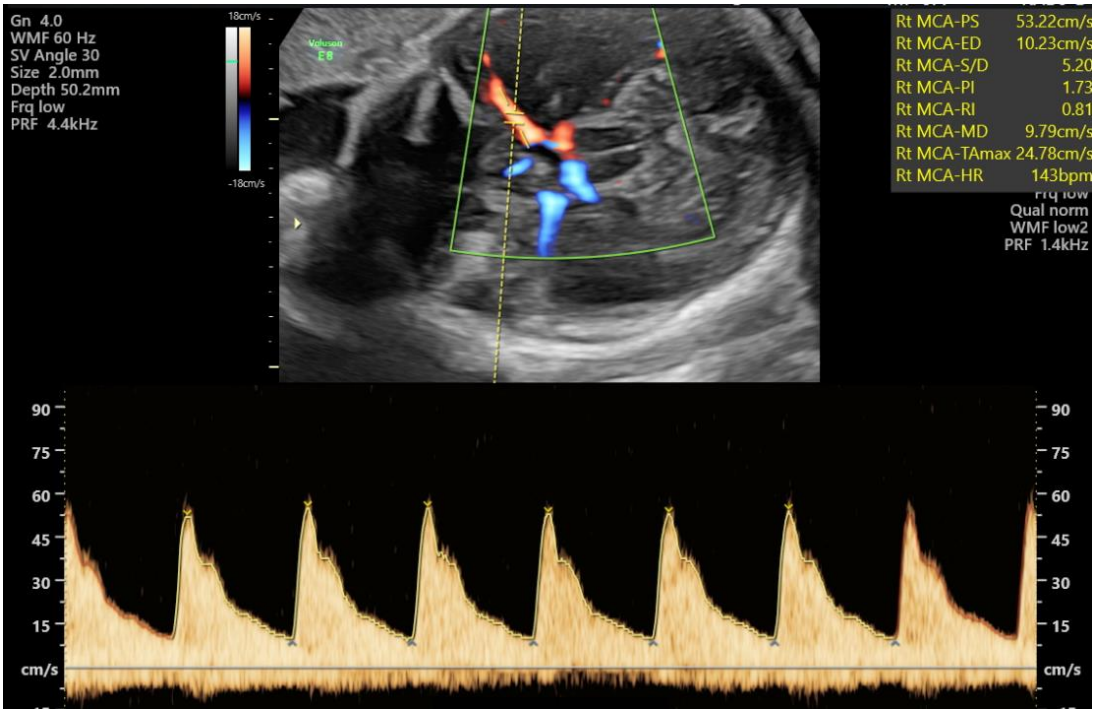
Figure 2.10 Umbilical Artery Doppler Waveform



Ultrasound image of a fetal umbilical artery Doppler waveform.

Abbreviations: Umb, umbilical artery; PS, peak systolic; ED, end diastolic; S/D, systolic/diastolic ratio; PI, pulsatility index; RI, resistance index; MD, mid diastole; TAmax, time averaged maximum velocity; HR, heart rate.

Figure 2.11 Middle Cerebral Artery Doppler Waveform



Ultrasound image of a fetal middle cerebral artery Doppler.

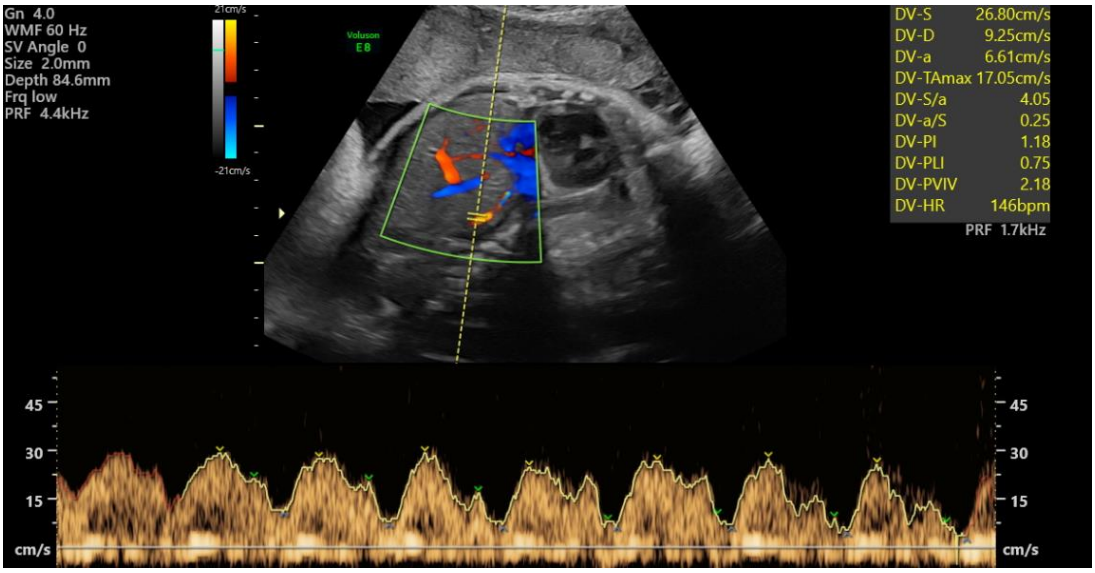
Abbreviations: PS, peak systolic; ED, end diastolic; S/D, systolic/diastolic ratio; PI, pulsatility index; RI, resistance index; MD mid diastole, TAmax, time averaged maximum velocity; HR, heart rate.

2.4.9 Ductus Venosus Doppler

The DV was identified in a mid-sagittal view at the isthmus, which is located close to the vessels' origin from the UV. Ultrasound colour Doppler mapping was used to identify the isthmus. This is often apparent in the DV due to aliasing that occurs here (270). The Doppler sample gate was kept as small as possible to reduce the risk of contamination of the true waveform from other adjacent vessels. A good quality triphasic waveform was acquired (*Figure 2.12*) and was firstly assessed qualitatively (such as positive, absent or reversed a-wave) and secondly by using the automatic machine function. Ratios for the measurements were calculated automatically as described below.

Pulsatility Index for Veins (PIV)	$\frac{\text{Systolic – End-diastolic Velocity (a)}}{\text{Time averaged maximum velocity}}$
Peak Velocity Index for Veins (PVIV)	$\frac{\text{Systolic – End-diastolic Velocity (a)}}{\text{Diastolic peak velocity}}$
Preload Index (PLI)	$\frac{\text{Systolic – Diastolic peak Velocity}}{\text{Systolic peak velocity}}$

Figure 2.12 Ductus Venosus Doppler Waveform



Ultrasound image of a fetal ductus venosus Doppler taken in the sagittal plane demonstrating a normal triphasic flow pattern.

Abbreviations: S, systolic wave; D, diastolic wave; A, pre-systolic wave, TAmax, time averaged maximum velocity; S/a, Ventricular systolic to active diastolic filling ratio; PI, pulsatility index, PLI, preload index, PVIV, peak velocity index for the vein; PIV, pulsatility index for the vein; HR, heart rate.

2.4.10 Fetal Pulmonary Artery Doppler

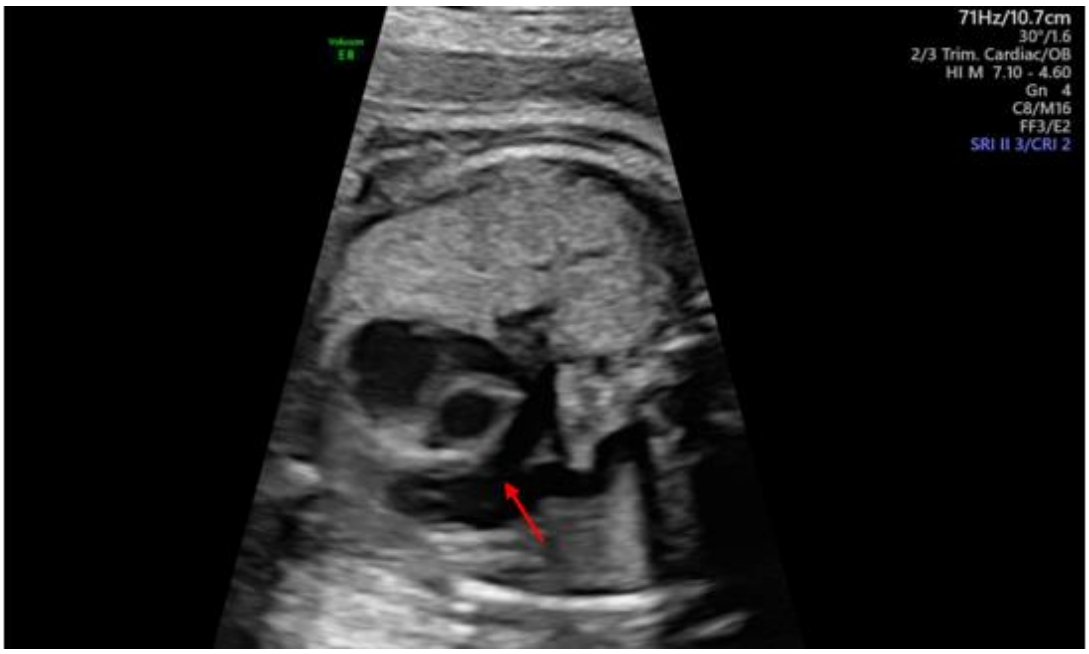
A systematic examination of the fetal heart was first performed according to an agreed protocol *(Table 2.1)* to exclude any major structural defect. A sequential segmental analysis of the atria, ventricles, great arteries and their connections was performed. The fetal PA was visualised by rotating the transducer from the four-chamber view to the short-axis view of the fetal heart. The pulmonary valves and the bifurcation of the right and left branches of the PA were identified *(Figure 2.13).* The distal branch PA (DPA) was located beyond the first bifurcation of the branch PA. The pulsed Doppler sample gate was placed at this point and away from the arterial walls. The image was enlarged as much as possible. The sample gate was adjusted to approximately 3mm. An angle of insonation of <15 degrees between the vessel and Doppler beam as assessed by colour Doppler was accepted for analysis. The blood flow waveform was displayed with a velocity range of 100cm/s and a sweep speed of 200mm/s. The Doppler velocity waveform of the fetal DPA was recorded and produced the characteristic waveform for analysis commonly referred to as the “spike and dome” pattern (*Figure 2.14*).

Table 2.1 Fetal Echocardiogram Examination Protocol

Fetal Echocardiogram assessment:

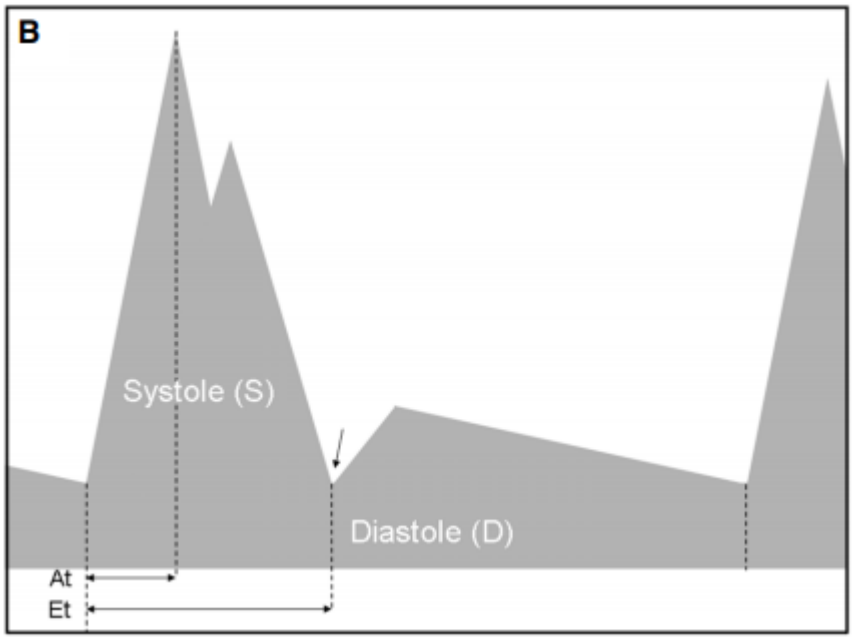
1. Image quality
2. Situs
3. Position of stomach
4. Heart size
5. Position of apex
6. Systemic veins
7. Pulmonary veins
8. Atrial septum
9. Flow at foramen ovale
10. Atrioventricular junction
11. Atrioventricular valve regurgitation
12. Ventricular septum
13. Ventricular function
14. Great artery connections
15. Arterial valve regurgitation
16. Branch pulmonary artery
17. Ductus arteriosus
18. Aortic arch
19. Side of arch
20. Subclavian arteries
21. Rhythm
22. Rate

Figure 2.13 Ultrasound Image of Branching Fetal Pulmonary Artery



Ultrasound image of the fetal branching pulmonary artery in an extended three vessel view. The red arrow marks the area where the Doppler velocimetry waveform was obtained, i.e. beyond the first bifurcation of the branch pulmonary artery.

Figure 2.14 Fetal Pulmonary Artery Doppler waveform illustration



The unique pulmonary artery Doppler waveform pattern is characterised by a rapid initial flow acceleration phase and an equally rapid deceleration phase, producing the characteristic needle-shaped systolic peak.

Abbreviations: S, systole; AT, acceleration time; ET, ejection time; T, Time; D, Diastole. Illustration taken from (226).

Differentiation of the PA waveform from the DA waveform was important to avoid error and to allow for accurate measurement. The fetal DPA waveform demonstrates a needle-like sharp systolic peak corresponding to ventricular systole, a small “notch” of reversed flow is also seen at the end of systole (231). The diastolic phase which has a lower velocity, begins at the ‘notch’ which is caused by the closure of the pulmonary valves. In contrast the DA Doppler waveform has its own characteristic pattern and is described in *Section 2.3.6*.

The specific PA measurements were averaged from the values obtained over three cardiac cycles and these included: The PSV which was defined as the maximum blood flow velocity reached during systole, the EDV and the time averaged velocity (TAV). The PA PI was measured as the difference between the peak systolic and end diastolic velocity divided by the TAV.

$$PI = (PSV-EDV)/TAV$$

The PA RI was calculated as follows: PA RI= (PSV-EDV)/PSV). The PA ejection time (ET) was defined as the time interval from the beginning to the end of ventricular systole. Pulmonary artery acceleration time (AT) was defined as the time from the initial increase in velocity (beginning of ventricular systole) to the time of peak velocity. The time velocity integral (TVI) was classified as the area under the velocity spectral envelop. All of the above indices were measured using a manual trace over three cardiac

cycles. All ultrasound data were recorded on the ultrasound software system (Viewpoint; MDI Viewpoint, Jacksonville, Florida).

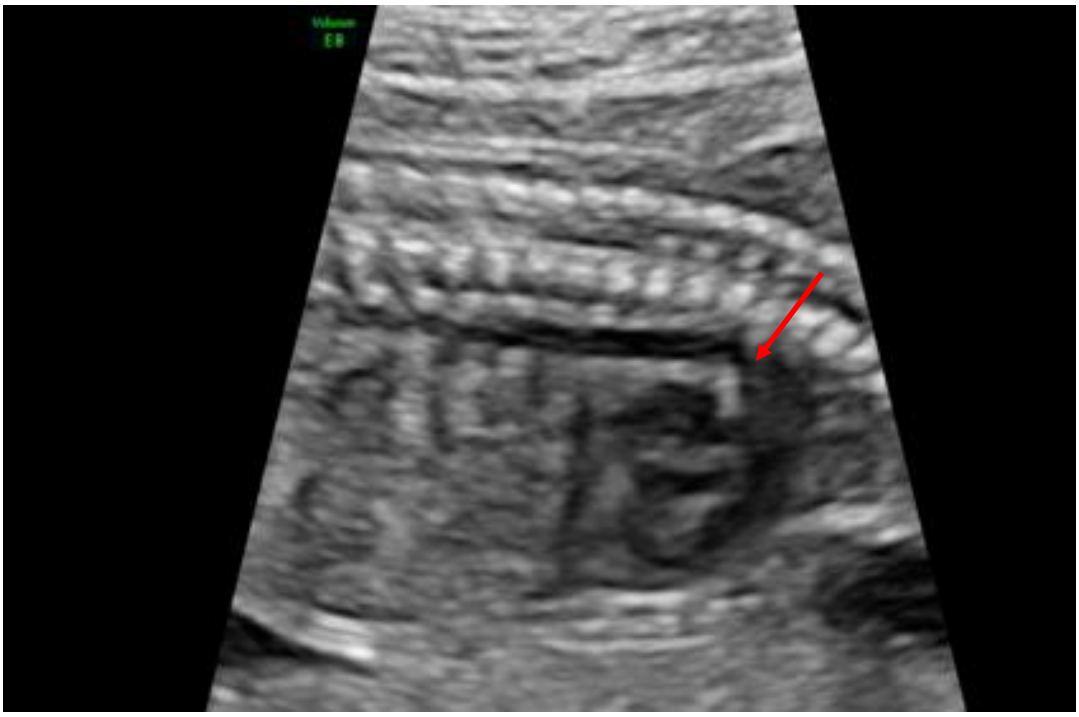
2.5 Other Fetal Cardiac Dopplers

2.5.1 Fetal Aortic Isthmus Doppler

Fetal AoI Doppler parameters were assessed in the LAA view *(Figure 2.15).* This view has been shown to provide reproducible Doppler parameters as well as the three vessel and tracheal sonographic plane (234). In the traditional LAA view the sample gate was placed just beyond the origin of the left subclavian artery, as previously described by Bonnin *et al* (336). Care was taken to avoid aliasing to reduce error in AoI Doppler measurement. This was insured by adjusting the colour Doppler maximal velocity setting to high velocities. In this setting, the fetal blood flow was homogeneous in colour and showed no aliasing. The high-pass filter was set at 50Hz and energy output levels were lower than 50mW/cm^2. The scanning plane was adjusted to obtain an insonation angle as close to 0° as possible, and always <15°, to reduce the possibility of artefacts from the aortic root or DA. After a minimum of three uniform AoI Doppler waveforms were obtained, the image was frozen and saved to the ultrasound machine for future analysis. The following measurements were averaged from the values obtained from the three best cardiac cycles, using a conventional computerised programme

linked to the equipment that calculated the PSV, EDV and time averaged maximum (TAmax) velocities, PI (PI=PSV-EDV/TAMXV) and RI (RI=PSV-EDV/PSV).

Figure 2.15 Fetal Aortic Isthmus Doppler

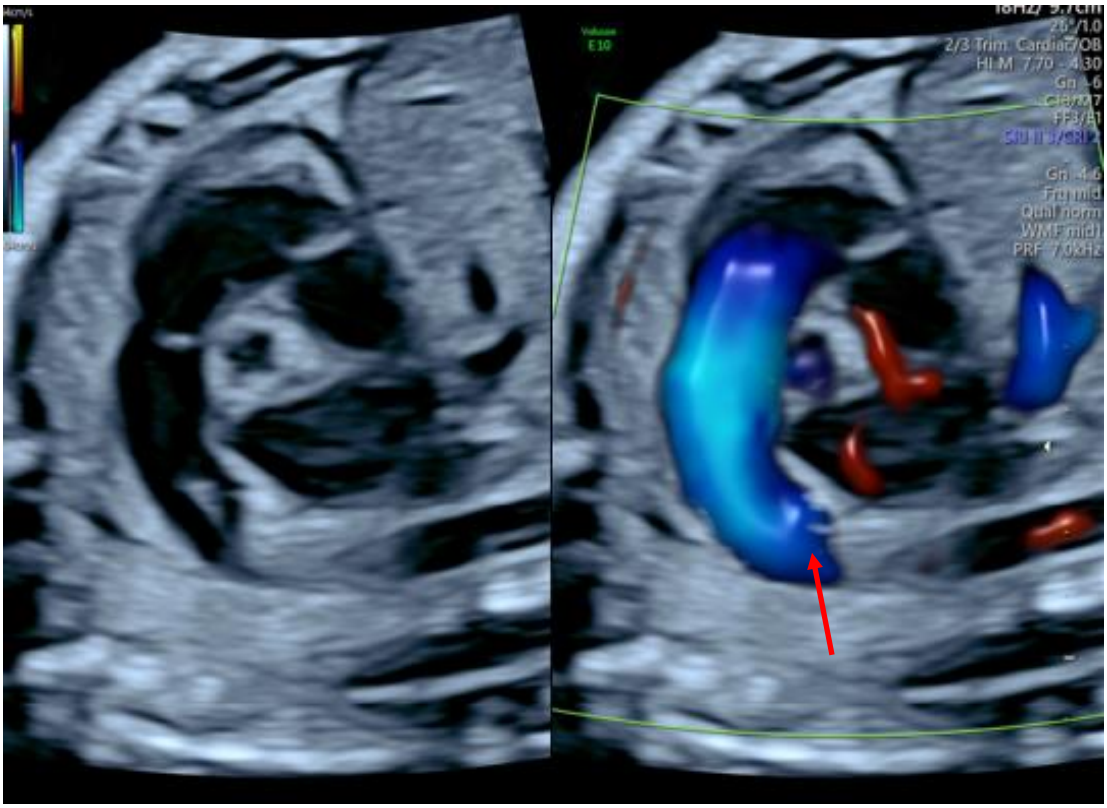


Longitudinal views of the aortic arch with the red arrow indicating the aortic isthmus (AoI) and the point at which the aortic isthmus Doppler waveform was obtained.

2.5.2 Fetal Ductus Arteriosus Doppler

The fetal DA Doppler waveform is characterized by a rounded, full, and triangular-shaped systolic blood flow pattern that is “dome-like” in appearance. Distinguishing it from the fetal PA Doppler was necessary for accurate acquisition of the waveform, which has a greater diastolic flow and peak velocity than the PA (337). The DA waveform can be obtained on ultrasound in either the three-vessel trachea view or the longitudinal ductal arch (LDA) view. The three-vessel tracheal view is typically obtained in the horizontal direction of the fetal body and therefore the angle between the DA and the descending aorta is relatively large. This is thought to reduce the likelihood of forming the smallest angle between the DA and the ultrasound beam (338). For this reason, all of the DA Doppler waveforms that we obtained were in the LDA view (*Figure 2.16*). This was obtained by slightly tilting the transducer from the right ventricular outflow tract (RVOT) view until the RVOT, major PA trunk and the DA were aligned. In the sagittal section of the DA arch, the characteristic ‘hockey stick’ shaped curve was clearly visualised (339). In the LDA view, the ultrasound transducer was manoeuvred to obtain the smallest angle between the DA blood flow and the ultrasound beam. The Doppler sampling gate was placed between the origin of the left PA and the beginning of the descending aorta. Measurements from the DA included PSV, EDV, PI, RI, and TAmax. Measurements were recorded by the automatic machine function. Reported values were averaged from three consecutive waveforms.

Figure 2.16 Longitudinal Ductal Arch View of the Ductus Arteriosus



The pulmonary artery as illustrated in this ultrasound image, arises from the anteriorly positioned right ventricle and courses towards the descending aorta. The ductal arch has a nearly perpendicular shape and resembles a hockey stick. The red arrow represents where the ductus arteriosus Doppler waveform was obtained.

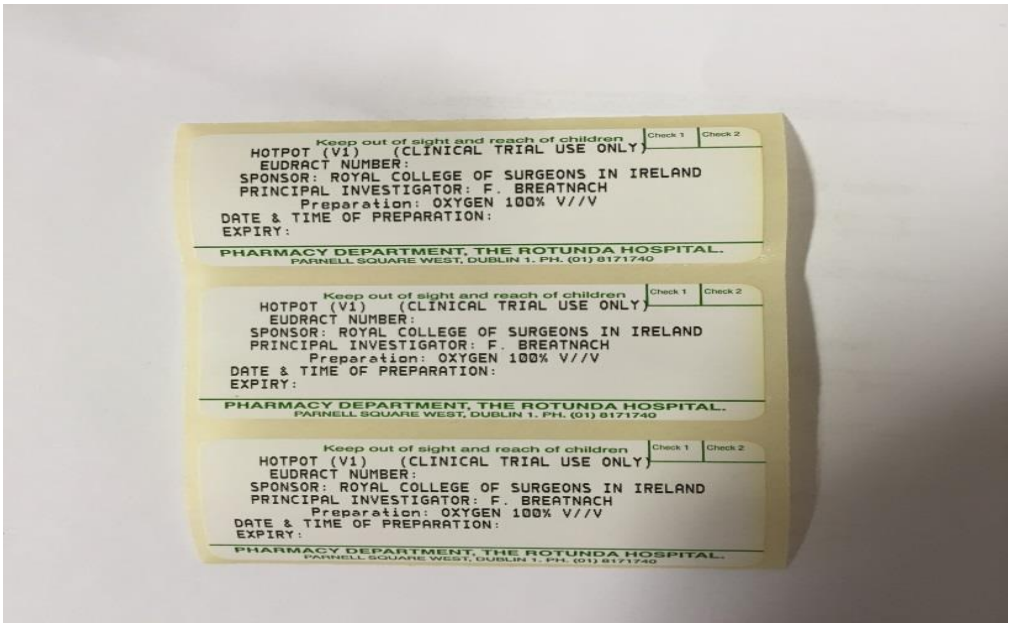
2.5.3 Oxygen Administration

Oxygen was administered to the participants while in a semi-recumbent position in the hospital ultrasound department. The ultrasound department in our hospital was equipped to administer oxygen to the participants, using a portable oxygen cylinder and disposable plastic non-rebreather masks. The capacity of a compressed oxygen cylinder was 400Litres. Oxygen was administered at a rate of 12L/min for a total duration of ten minutes via a non-rebreather face mask. The participant was asked to put on the face mask and to adjust the strings at the side of the mask until comfortable *(Figure 2.17).* They were then asked if they felt a tight seal of the mask around their nose and mouth. All face masks were checked for correct positioning before oxygen administration commenced. Oxygen was prescribed in the patient's drug kardex and a specifically designed HOTPOT study oxygen drug administration sticker was placed in the patient's drug kardex to distinguish this from any other prescription of oxygen and specifying that it was for research purposes only *(Figure 2.18).* Immediately following MH, a repeat fetal echocardiogram was performed, and all Doppler recordings were repeated. Each fetus served as its own control.

For non-pregnant participants, oxygen was administered in a semi-recumbent position at a rate of 12L/min via a non-rebreather mask for a total of ten minutes. These participants did not have a medical drug kardex and therefore prescription of oxygen was not performed for this cohort.

Figure 2.17 Non Re-breather Oxygen Face Mask

Figure 2.18 Drug Kardex Label for Oxygen Administration



Investigational Medicinal Product – Oxygen

The trade name of the medicinal product is medical oxygen. The name of the active substance is oxygen. The formulation is 100% V/V inhalational gas. The marketing authorisation number in Ireland is PA1357/001/001. The marketing authorisation holder is Industrial Pressure testing Ltd. The dose administered was 60% FiO_2. Oxygen cylinders were stored under cover in the ultrasound department, kept dry and clean, and not subjected to extremes of heat or cold. They were stored separately from other medical and non-medical cylinders and away from any combustible materials. Warning notices prohibiting smoking and naked flames were posted clearly in ultrasound cubicle where all study procedures involving oxygen administration took place. The non-rebreathing face mask has an oxygen reservoir bag attached *(Figure 2.17),* which was used to deliver high concentrations of oxygen to the study participants. The one-way valve functions to divert flow of oxygen into the reservoir bag during expiration. The contents of the reservoir bag together with the high flow of oxygen, result in minimal entrainment of air and an inspired oxygen concentration of approximately 60%-80%. The valve also prevents exhaled gases from entering the reservoir bag.

2.5.4 Acceptability Questionnaire

Following the administration of MH a verbal questionnaire was administered to all participants. It comprised of three questions:

1. Was the oxygen test comfortable?

2. Was the overall test too long?

3. Would you undergo this test again?

2.5.5 Neonatal Echocardiogram

A functional neonatal echocardiogram was performed within the first 24 hours of life by one of three senior neonatal clinical research collaborators. The echocardiography machine used in the study is primarily dedicated to research and was accessible 24 hours a day. The person performing the neonatal echocardiogram was not aware of the prenatal response to hyperoxygenation. Neonatal data collection included GA at delivery in weeks, timing of echocardiogram in hours and birth weight in grams. Echocardiography was carried out using a Vivid S6 echocardiography machine and a 7MHz neonatal probe (GE Medical, Milwaukee, USA). Studies were conducted during a resting state in accordance with recent guidelines and CHD was evaluated for during the first scan (340). Data were stored as raw Digital Imaging and Communications in Medicine (DICOM) images in an archiving system (EchoPac, General Electric, version 112

revision 1.3) and analysis of all the echocardiography parameters was carried out by a single investigator who was blinded to the results of the fetal Doppler ultrasounds. The following echocardiography parameters were measured using previously described methods (341), left ventricular (LV) length measured at end diastole; mitral valve annular diameter; left ventricular output (LVO, ml/kg/min); ejection fraction using Simpson's Biplane method (EF, %); mitral valve inflow velocities and velocity time index, PDA diameter (mm) measured in 2D at the pulmonary end; diastolic and systolic flow velocity across the PDA; flow pattern across the duct; pulmonary artery acceleration time (PAAT); and right ventricular (RV) end systolic pressure measured using the tricuspid valve regurgitant jet. Right ventricular systolic time interval measurements are seen in *Figure 2.19.* The neonatal LVO was measured as:

Neonatal LVO =

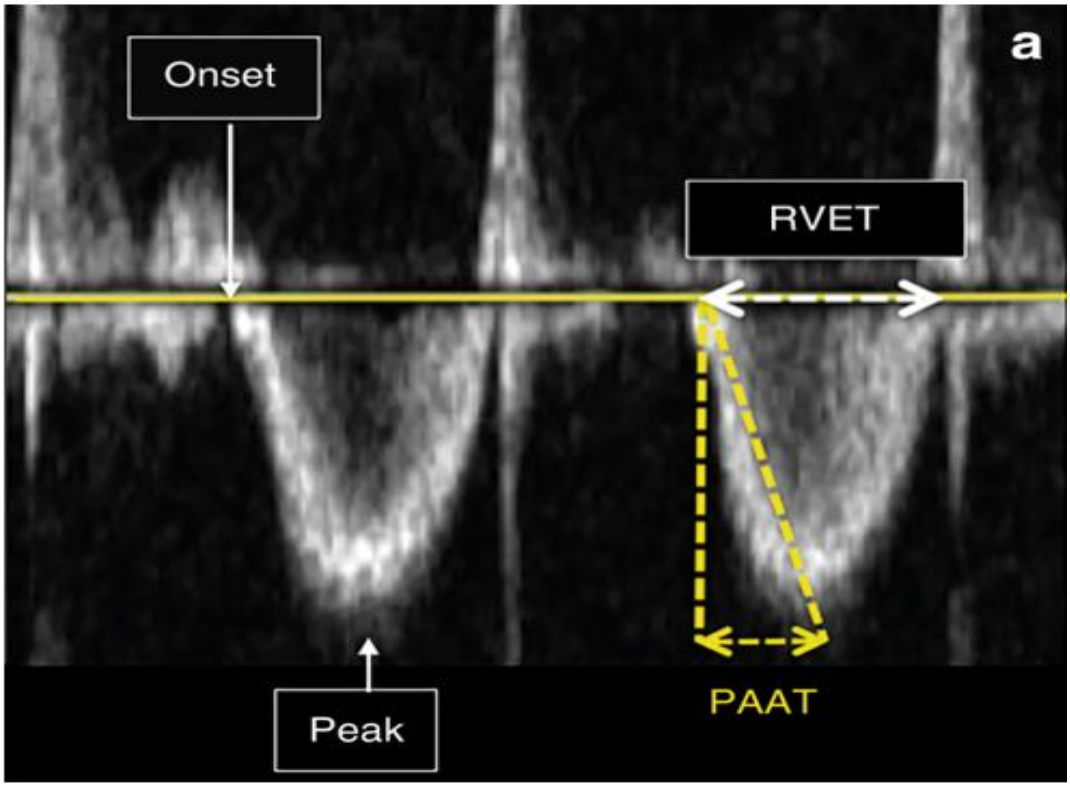
Cross sectional aortic area (cm^2) x flow velocity integral (cm) x heart rate per minute

Neonatal EF was measured as:

Neonatal EF (%) =

LVEDV – (LVSV x LVEDV) X 100

Figure 2.19 Right ventricular systolic time intervals



This figure measuring right ventricular systolic time intervals demonstrates normal pulmonary artery pressure and pulmonary vascular resistance.

Abbreviations: RVET, right ventricular ejection time; PAAT, pulmonary artery acceleration time (Taken from De Boode et al (340)).

2.6 Pregnancy and Paediatric follow-up

The Rotunda Hospital iPIMS software was used to monitor when the patient had delivered their baby or if they had been admitted to the hospital for any reason. Once the patient had delivered, they were offered the neonatal echocardiogram within 24 hours of life. They had previously consented to having this procedure, but all participants were asked for verbal consent again in the postnatal period. Participating in the study did not affect their routine postnatal care and they were discharged home from hospital when deemed clinically well by the hospital clinicians in charge of their routine care. Neonatal follow-up was undertaken in the paediatric outpatient department six weeks following discharge from hospital for babies who were deemed to require hospital follow-up by the paediatrician on discharge from hospital. For babies not deemed to require hospital follow-up by the paediatrician, follow-up was performed over the telephone. At this review data were collected in relation to neonatal course and wellbeing. As this was a pilot study of a low risk population, an additional review following the six week postnatal review was not deemed necessary.

2.7 Data Collection and Retention

All data were collected on a single encrypted database on a password protected study laptop (NICOM® data) and on a desktop computer (NICOM® and non-NICOM® data). Participant details were recorded under a specific study number, rather than by name or any other identifier. Access to the database was password protected to ensure data protection. The maternal and neonatal data collection was fully completed at the time of the six-week postnatal review. All fetal Doppler recordings and ultrasound measurements were stored on the ultrasound machine for further analysis and for data safety monitoring (DSM) purposes. Source documents for this study included hospital records, ultrasound procedure reports, data collection forms and ultrasound images with incorporated measurement indices. These documents were used to enter data onto the case report files (CRFs). Data reported on the CRF was derived from source documents and was verified as being consistent with the source documents at the DSM meetings. All documents were stored safely in confidential conditions. On all study-specific documents other than the signed consent, the subject was referred to by the study subject identification number. The subjects were identified by a study specific subject number in the database. All records and documents will be maintained by the investigator for a period of at least two years.

Data Monitoring

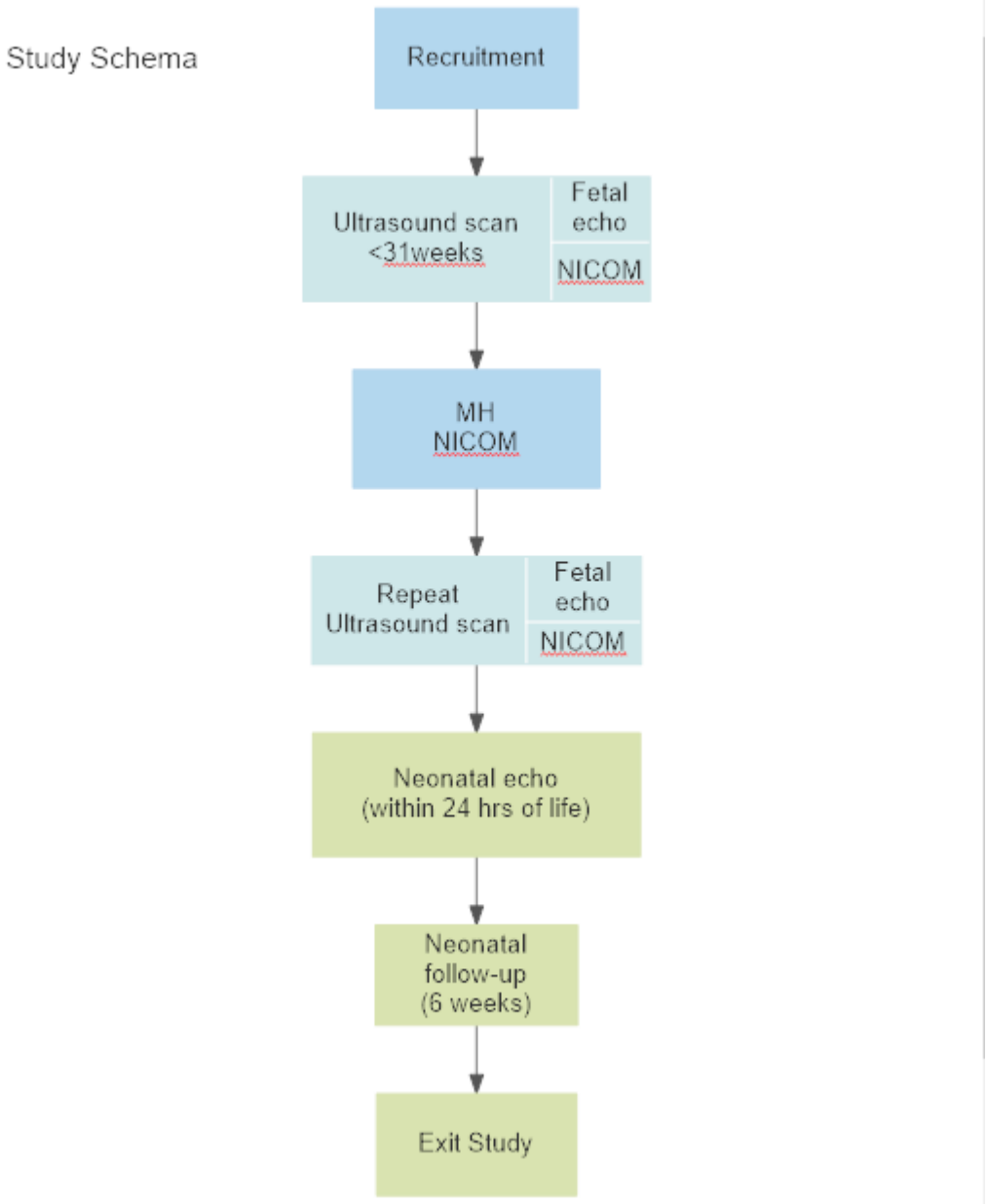
The study was conducted in accordance with the most recently approved protocol and by the International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) good clinical practice (GCP) guideline. It was also conducted in accordance with general data protection regulations (GDPR) and standard operating procedures.

The data monitor for the study was Ms Mandy Jackson, a Quality and Regulatory Affairs manager with the Royal College of Surgeons in Ireland (RCSI). All data entries were source verified. A number of DSM meetings were held between Ms Mandy Jackson and the study team and any issues in relation to data acquisition, storage and study safety were addressed. See log of DSM meetings.

Data Safety Monitoring (DSM) Meetings

SIV 21st November 2016
27th July 2017
28th September 2017
27th March 2018
28th June 2018
Study complete September 2019
Close-out visit 26th February 2020

2.8 HOTPOT Study Schema



2.9 Equipment and Resources

The following resources were available to the study group:

- The use of one NICOM® device (Cheetah, Medical, Maidenhead, Berkshire, United Kingdom)
- 100 sets of NICOM® electrode sensors to perform haemodynamic assessments on all participants.
- Use of the Voluson E8 ultrasound machine GE Medical, Milwaukee) in the ultrasound department. A dedicated slot on a Monday afternoon (half-day session) was granted for the research scans.
- Use of the Rotunda paediatric outpatient department for the postnatal reviews.
- Administrative support from the Rotunda Hospital staff in accessing all maternal and neonatal charts.
- Use of the Vivid S6 echocardiography system (GE Medical, Milwaukee) courtesy of the Neonatal Research Department.

2.10 Ethics and Consent

The study was approved by the National Maternity Hospital, Dublin, Ireland, Research Ethics Committee (REC) in September 2016. This is a national ethics committee that grants approval for studies that are conducted in maternity units in Ireland. Participants were provided with an information leaflet and a copy of the maternal consent form prior to attending the ultrasound department. This gave participants adequate time to make an informed decision on partaking in the study and to give informed consent. All participants completed a written informed consent prior to enrolment in the study. This included full disclosure of the nature, risks, and benefits of participation in the study. Consent was taken by the study investigator (A.M) at the participant's recruitment visit to the ultrasound department when they attended for the hyperoxygenation test. Prior to any study-related screening procedures being performed, the informed consent statement was reviewed, signed, and dated by the participant and counter signed by the investigator. The study was also approved by the Health Products Regulatory Authority (HPRA) in Ireland.

2.11 Funding

This project was supported by the Rotunda Foundation (formerly known as Friends of the Rotunda), Pillar Room, Rotunda Hospital, Parnell Street, Dublin 2. The Rotunda Foundation is a registered charity (CHY20091) and granted €26,500 in funding for the HOTPOT study.

Applicants: Dr Ann McHugh, Dr Orla Franklin, Prof Afif El-Khuffash and Prof Fionnuala Breathnach.

2.12 Study Sponsor and Insurer

Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2.

2.13 Study Registration

EnduraCT Number 2016-003181-12

3 CHAPTER 3 Results

3.1 Demographics

There were 46 pregnant and 20 non-pregnant women recruited to this study with a median age of 33 [26-38] and 32 [28-37] years respectively (p=0.82).There were no differences between the mean BMI in the pregnant group measured at the booking visit (<14 weeks gestation) and the non-pregnant group (26.4 ± 4.1 kg/m^2 vs. 24.5 ± 3.6 kg/m^2; p=0.08). The mean BMI in the pregnant group in the third trimester was 29.9 ± 5.4 kg/m^2. The majority of the 46 pregnant participants were multiparous (61%, n=28/46). The median gestational age at the time of recruitment was 35.5 weeks [33.4-36.9]. A total of 25 women (54%, n=25/46) received antenatal corticosteroids during their antenatal course, prior to recruitment to the study. Indications for the administration of antenatal corticosteroids included PPROM, (32%, n=8/25); bleeding placenta praevia, (20%, n=5/25); unspecified vaginal bleeding (20%, n=5/25); elective CS ≤ 38 weeks GA, (16%, n=4/25); threatened preterm labour, (8%, n=2/25); and undocumented, (4%, n=1/25). Demographic data from all pregnant participants are presented in *Table 3.1.*

Table 3.1 Pregnant Participant Demographic Data

Demographic data in the third trimester (n = 46)	
Age (years)	33 [26-38]
Gestational age (weeks) GA 31-33+6 GA 34-40	35.5 [33.4-36.9] n=14 (30%) n=32 (70%)
Weight (kg)	83 ± 17
Height (cm)	166 ±7
BMI (kg/m2)	29.9 ± 5.4
Parity 0 1 2 3 ≥ 4	 18 (39%) 16 (35%) 9 (20%) 2 (4%) 1 (2%)
Caucasian	43 (93%)
Haemoglobin in third trimester (g/dL)	11.9 ± 0.92

Abbreviations: BMI, Body Mass Index; GA, Gestational age.

Figures presented as N (%), median [IQR] or mean ± SD.

3.2 Recruitment

A total of 55 pregnant study participants and 20 non-pregnant participants were screened, met inclusion criteria, and were approached to take part in the study *(Figure 3.1).* All potential study participants were given an information leaflet on the study. All non-pregnant participants consented to the study and were recruited (100%, n=20/20). Four women (5%, n=4/75) who met eligibility criteria were approached and declined participation in the study. Of this cohort, two had a prenatal diagnosis of a fetal VSD (Group C) and two had no fetal structural abnormality and were planned to be recruited to the control group (group D). Additionally, five women (6.7%, n= 5/75) with a diagnosis of a mid-trimester PPROM (Group A) agreed to partake in the study. However, all delivered prior to the recruitment eligibility gestation of ≥31 weeks. This resulted in 66 participants being consented and recruited to the study. Of all participants, 46 were pregnant and 20 were not pregnant. Participants consented for the study at the time of their appointment and ultrasound scan. The consent form was put into the participant's medical chart (pregnant group) and a copy of the form was filed with the data collection working site file.

3.2.1 Recruitment By Group

Group A: *Fetuses at risk of pulmonary hypoplasia: mid-trimester PPROM, CDH, skeletal dysplasia, and persistent oligohydramnios.*

Eleven participants were recruited to this group (16.7%, n=11/66). All had a diagnosis of PPROM (100%, n=11/11). The median GA at which PPROM was confirmed was 30.5 [20.5-33.4] weeks. There was one mid-trimester PPROM (20.5 weeks gestation) recruited to the group. The median length of time from PPROM to delivery was 20 [11-31] days. As previously described a further five potential participants with mid-trimester PPROM delivered before recruitment. All other participants had a PPROM confirmed in the third trimester (range 31.4-34.5 week's gestation) and were confirmed as having persistent oligohydramnios on ultrasound.

Group B: *Women attending for scheduled CS prior to 38 weeks' GA*

Twelve participants (18.2%, n=12/66) were recruited to this group. The median GA at delivery in this group was 37.0 weeks [36.8 – 37.2]. The majority (92%, n=11/12) of participants in this group underwent a CS at less than 38 weeks gestation due to an antenatal diagnosis of a placenta praevia.

Group C: *Fetuses with a prenatal diagnosis of a moderate or severe perimembranous VSD or AVSD in the absence of other structural heart disease including fetuses with Trisomy 21.*

Three patients were recruited to this group (4.5%, n=3/66). Two additional women meeting the group C criteria were approached and declined participation in the study. None of the cases had an antenatal diagnosis of Trisomy 21. I performed a retrospective review of all moderate and severe perimembranous VSD and AVSD cases, in the absence of any other structural abnormality and in normally grown fetuses attending the Rotunda Hospital over the study period. Nine cases meeting this groups eligibility criteria were identified, including the five cases that were screened in this study.

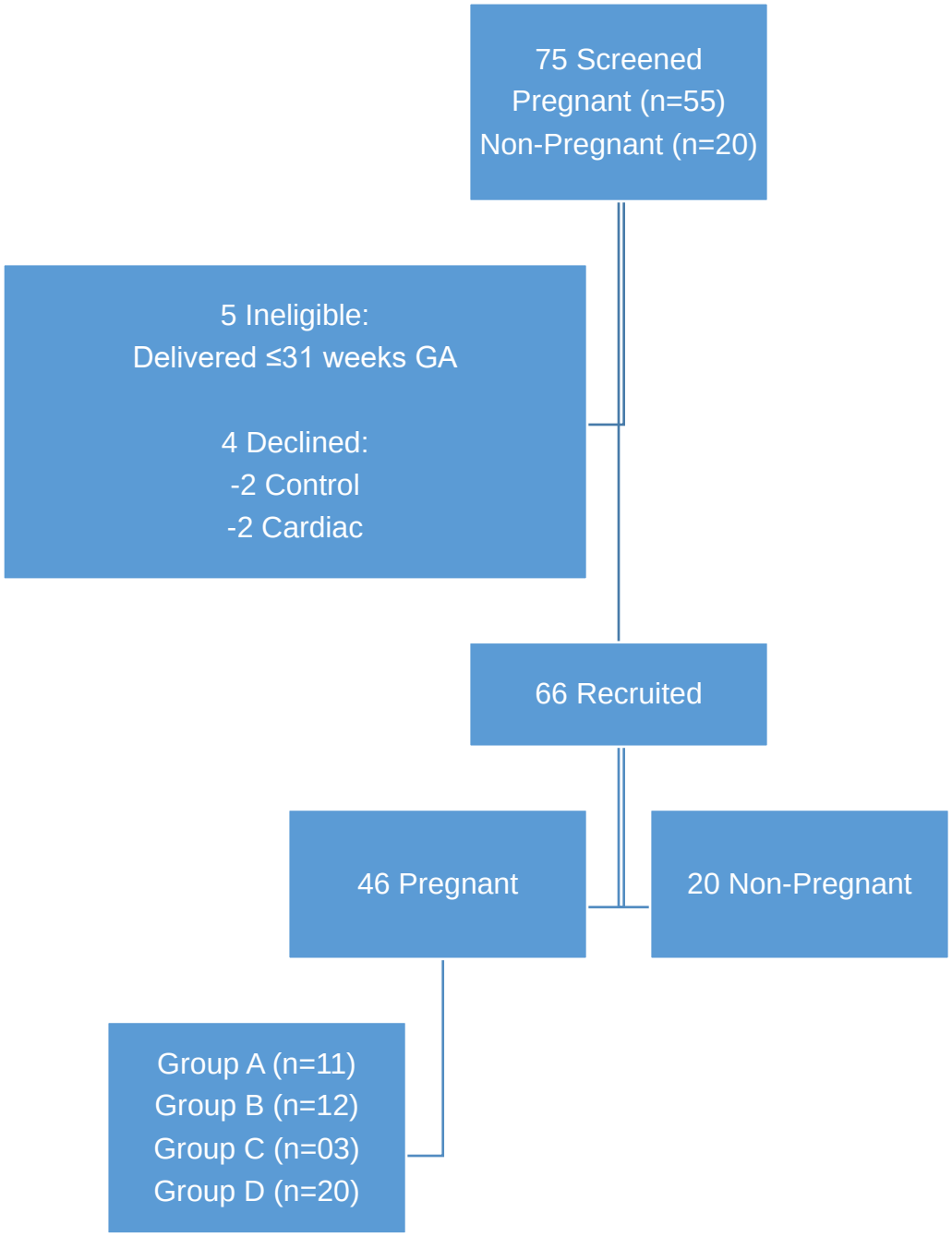
Group D: *A group of gestation-matched uncomplicated singleton pregnancies serving as a control group.*

There were 20 pregnant controls recruited to this group. As previously described, only two screened subjects refused participation in the study.

Group E: *Non-pregnant participants*

There were 20 non-pregnant participants approached and offered participation in the study. All were given an appointment to attend the ultrasound department for the study visit. All 20 participants (100%, n=20/20) attended and were recruited to the study.

Figure 3.1 Study Population Profile



3.3 Haemodynamics

Baseline haemodynamic measurements in the pregnant and non-pregnant groups are illustrated in *Table 3.2*. Cardiac Index (CI) is a measure of CO indexed to body surface area (342). At baseline, there was a significantly higher mean CO (6.3 ± 1.1L/min vs. 4.9 ± 1.1L/min, p=0.001) and concomitantly higher mean CI (3.3 ± 0.5L/min/m^2 vs. 2.8 ± 0.6 L/min/m^2, p=0.004) in the pregnant group versus the non-pregnant group. The baseline mean HR in the pregnant group was higher than in the non-pregnant group (87 beats per minute (bpm) vs. 72bpm, p=0.001). There was a lower baseline mean SVR in the pregnant group compared with the non-pregnant group (1236 ± 286dynes/sec/cm^{-5} vs.1509 ± 312dynes/sec/cm^{-5}, p=0.002). We found no significant differences in baseline mean SV (73 ± 13mL vs. 68 ± 13mL, p=0.16) or SBP (121 ± 17mmHg vs.114 ± 8mmHg, p=0.083) between the two groups.

Table 3.2 Baseline Haemodynamic Measurements in Pregnant vs. Non-Pregnant Subjects

Baseline measurements	**Pregnant (n=46)**	**Non-Pregnant (n=20)**	**p-value**
CO (L/min)	6.3 ± 1.1	4.9 ± 1.1	0.001
CI (L/min/m^2)	3.3 ± 0.5	2.8 ± 0.6	0.004
SVR (dynes/sec/cm^{-5})	1236 ± 286	1509 ± 312	0.002
SV (mL)	73 ± 13	68 ± 13	0.16
HR (bpm)	87 ± 10	72 ± 9	0.001
SBP (mmHg)	121 ± 17	114 ± 8	0.083
DBP (mmHg)	78 ± 10	76 ± 7	0.14

Haemodynamic measurements in pregnant and non-pregnant participants at baseline.

Values displayed as means ± SD

Abbreviations: CO, cardiac output; CI cardiac index; SVR, systemic vascular resistance; SV, stroke volume; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

In the pregnant group there was a fall in mean CO and CI over the course of the hyperoxygenation exposure time, coupled with a rise in mean SVR, with no recovery by ten minutes following cessation of hyperoxygenation (*Figure 3.2*). The mean CO in the pregnant group decreased from a baseline of 6.3 ± 1.1L/min (Timepoint 1) to 6.1 ± 1.0L/min at ten minutes of hyperoxygenation (Timepoint 2) and continued to decrease to 5.7 ± 1.0L/min at ten minutes following the cessation of hyperoxygenation (Timepoint 3) (p=0.008).

A similar pattern was evident in the CI measurements in the pregnant group, where CI decreased from a baseline mean of 3.3 ± 0.5L/min/m^2 to 3.2 ± 0.6L/min/m^2 at ten minutes of hyperoxygenation and decreased again to 3.0 ± 0.5L/min/m^2 at ten minutes post cessation of hyperoxygenation (p=0.005). Mean SVR increased in the pregnant group during hyperoxygenation and continued to increase despite cessation of hyperoxygenation ten minutes prior (1236 ± 286dynes/sec/cm^{-5} to 1401 ± 301dynes/sec/cm^{-5}, p=0.009) (*Figure 3.2*).

Figure 3.2 Changes in Haemodynamics Over Time in Pregnant vs. Non-Pregnant Subjects

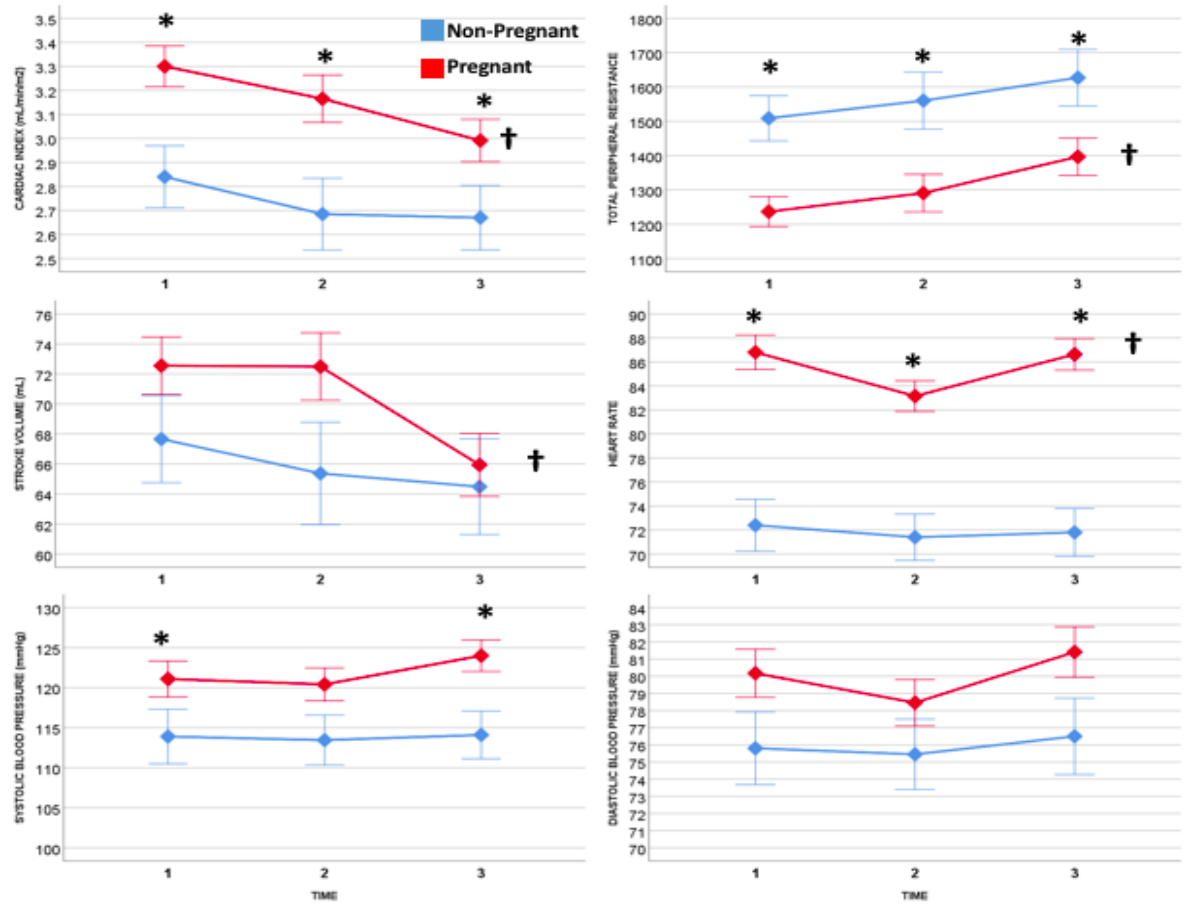


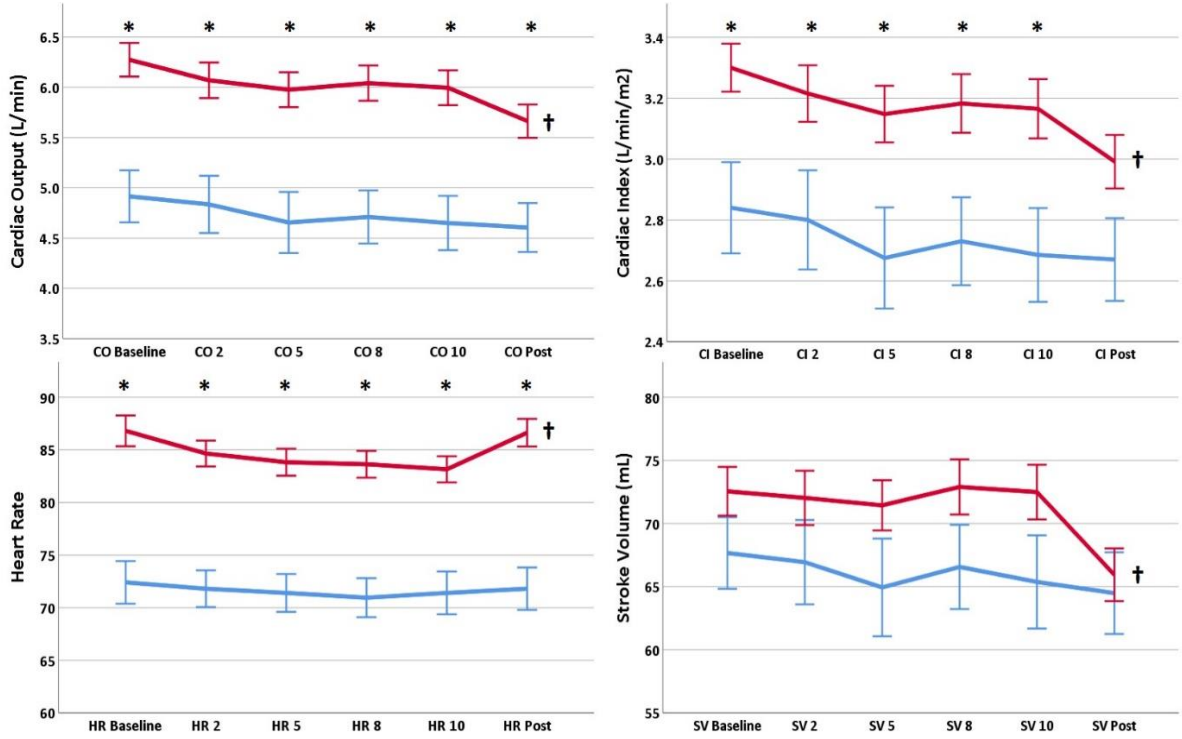
Figure1
*** = p <0.05 between groups at time point**
† = p <0.05 within group Time 3 vs. Time 1

Changes in cardiac index (CI), total peripheral resistance (TPR), stoke volume (SV), heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in pregnant versus non-pregnant subjects.

Time points refer to the following: Time 1 (before the administration of hyperoxygenation), Time 2 (at ten minutes of hyperoxygenation, Time 3 (ten minutes following the cessation of hyperoxygenation).

Mean maternal HR decreased from 87 ± 10bpm to 83 ± 8bpm (p=0.04) during hyperoxygenation returning to baseline levels (87 ± 10bpm) by 10 minutes post-cessation of hyperoxygenation. There was a decrease in mean SV post hyperoxygenation in the pregnant group from 73 ± 13mL to 68 ± 9mL (p=0.003), with no accompanying change in mean systolic or diastolic BP. In the non-pregnant group, there was no significant change in the mean CI (2.8 ± 0.6L/min/m^2 vs. 2.7 ± 0.6L/min/m^2, p= 0.60), SVR (1509 ± 312dynes/sec/cm^{-5} vs. 1560 ± 427dynes/sec/cm^{-5}, p=0.67), SV (68 ± 13mL vs. 65 ± 11mL, p= 0.53), systolic BP (114 ± 8mmHg vs. 113 ± 10mmHg, p=0.73) or diastolic BP (76 ± 7mmHg vs. 75± 6mmHg, p= 0.63) in response to hyperoxygenation. In the non-pregnant group, there was no change in mean HR (72 ± 9bpm vs. 71 ± 9bpm, p=0.72) over time. Serial changes in mean CO, CI, HR and SV at two, five, eight and ten minutes of hyperoxygenation are demonstrated in *Figure 3.3.*

Figure 3.3 Serial Cardiac Output, Cardiac Index, Heart Rate, and Stroke Volume in Pregnant vs. Non-Pregnant Subjects



(Pregnant - Red, Non-Pregnant - Blue)

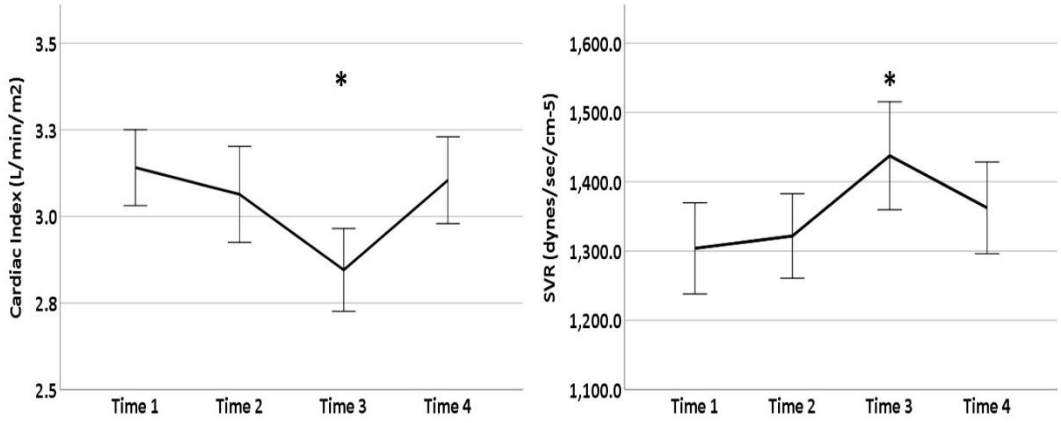
**= significant difference between groups at that time point*

†= significant change over time within group

Changes in cardiac output (CO), cardiac index (CI), heart rate (HR) and stroke volume (SV) in pregnant versus non-pregnant subjects at baseline, 2 minutes, 5 minutes, 8 minutes, and 10 minutes of hyperoxygenation. Post refers to Time 3 - ten minutes post the cessation of hyperoxygenation.

Following an interim analysis of our maternal data (n=24), it was decided that the NICOM® monitoring would be extended in any further pregnant study participant. A subset of 22 pregnant participants (48%, n=22/46) therefore underwent NICOM® monitoring at timepoint 4 which was at 30 minutes following the cessation of hyperoxygenation. At timepoint 4 the mean CI and SVR measurements had returned to baseline levels (3.2 ± 0.56L/min/m^2 vs. 3.1 ± 0.76L/min/m^2, p=0.58 and 1305 ± 234 dynes/sec/cm^{-5} vs.1360 ± 28 dynes/sec/cm^{-5}, p=0.49) *(Figure 3.4).*

Figure 3.4 Changes in Maternal Cardiac Index and Systemic Vascular Resistance in Response to Maternal Hyperoxygenation at Timepoint 4 (n=22)



** = significant change over time*

Abbreviations: SVR, systemic vascular resistance

Time 1, baseline; Time 2, at ten minutes of hyperoxygenation; Time 3, ten minutes post cessation of hyperoxygenation; Time 4, 30 minutes post cessation of hyperoxygenation.

Results from a subset of (n=22) pregnant subjects. Time 4 represents 30 minutes post the cessation of hyperoxygenation. CI and SVR levels have returned to baseline levels.

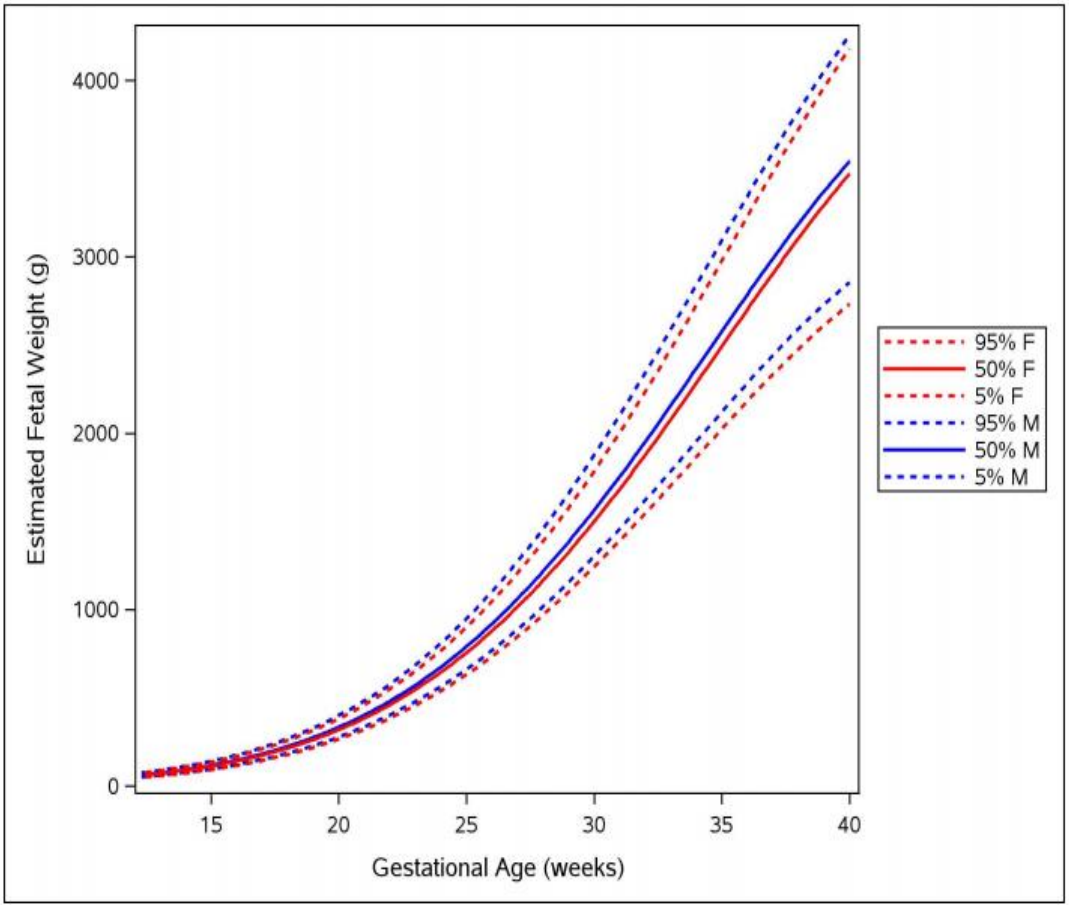
3.4 Ultrasound Assessments

3.4.1 Fetal Biometry

All participants (100%, n=66/66) had previously undergone a dating scan within the Rotunda Hospital and had their estimated due date defined. Gestational age was calculated from the sonographic measurement of the fetal CRL (326) as previously described in *Section 2.4.4.*

Fetal biometry measurements were obtained for all pregnant participants (100%, n=46/46). The ultrasound measurements obtained included the BPD, HC, AC, and FL. The EFW was calculated as previously described in *Section 2.4.4.* Each fetus was appropriately grown for GA (all between the 10th and 90th percentile on the fetal growth curve *(Figure 3.5).* The median GA at the time of the ultrasound assessment was 35.5 weeks [33.4-36.9]. The mean EFW was 2660g ± 626g at the time of the sonographic assessment. In the majority of cases (78%, n=36/46) amniotic fluid volumes based on a single DVP were within the normal range (4.8cm ± 1.8cm). In ten fetuses (22%, n=10/46) recruited to Group A, persistent oligohydramnios was confirmed by a single DVP of <2cm on ultrasound. All fetuses had a normal HR at first assessment and were in regular sinus rhythm. The mean fetal HR did not change significantly in response to hyperoxygenation (140 ± 12bpm vs. 136.8 ± 8bpm, p=0.08).

Figure 3.5 Normal Fetal Growth Curve



Estimated fetal weight during gestational weeks 14-40.

Abbreviations: F female; M, male

Taken from Kiserud et al (343).

3.4.2 Pulmonary Artery Doppler

Successful acquisition of PA Doppler indices were achieved in all participants (100%, n=46/46) There were no changes in the mean PA PSV at baseline and following ten minutes of MH (60.6 ± 11.4cm/s vs. 59.2 ± 14.1cm/s, p=0.60). The mean PA EDV did not change following MH (7.75 ± 2.2cm/s vs. 8.01 ± 2.1cm/s, p=0.56) (*Table 3.3*). Both the mean PA TAV and mean PA TVI increased in response to MH (16.18 ± 2.5cm/s vs.18.61 ± 4.9cm/s, p=0.004 and 5.3 ± 2.1cm vs. 6.2 ± 1.7cm, p=0.026 respectively). The mean PA PI decreased following MH from 2.47 ± 0.36 to 2.08 ± 0.33, (p=0.0001). The mean decrease in PA PI following MH was 21% (Range 9-36%). The PA PI response to MH for each individual participant is illustrated in *Figure 3.6*. The mean PA RI decreased following MH from 0.86 ± 0.05 to 0.78 ± 0.09, (p=0.0001) (*Table 3.3*).

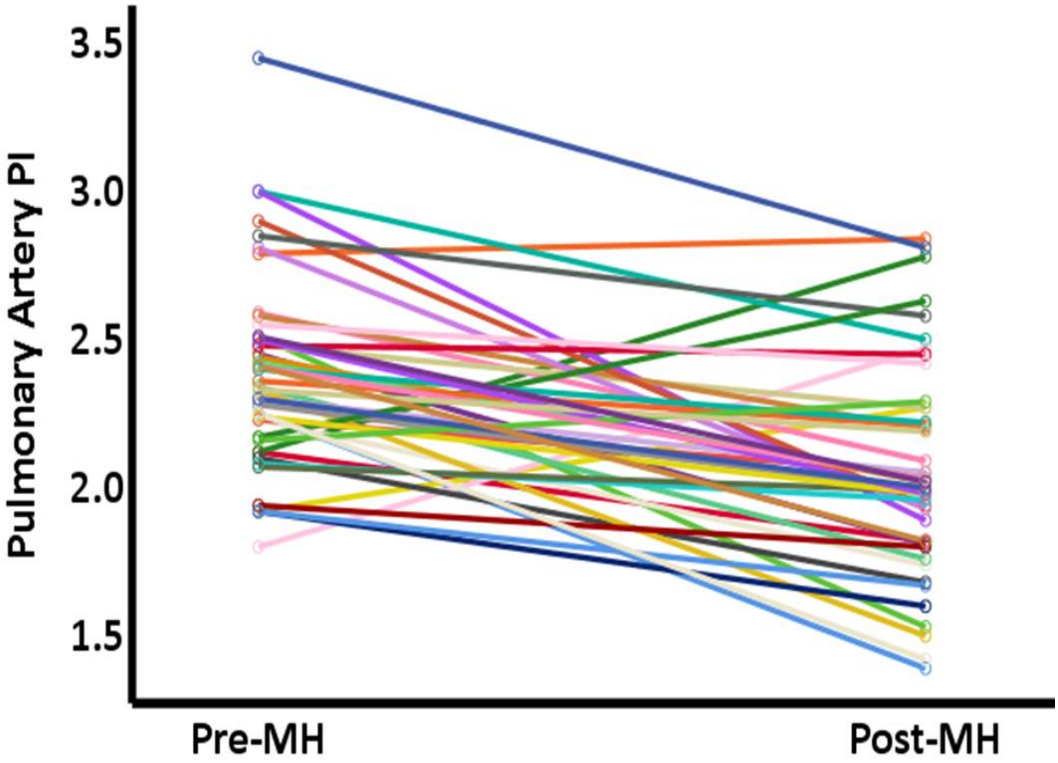
There was an increase in median PA AT (from 43ms [40-47] to 57ms [47-60], p=0.005) and PA ET (from 177ms [163-183] to 187ms [177-207], p=0.005), leading to an increase in AT:ET from 0.25 [0.24–0.28] to 0.32 [0.26– 0.34], p=0.005, indicating a fall in PVR following MH *(Figure 3.7).*

Table 3.3 Pulmonary Artery Doppler Changes Before and After Maternal Hyperoxygenation

Doppler measurements	**Pre MH**	**Post MH**	**p-value**
PA PSV cm/s	60.62 ± 11.4	59.2 ± 14.1	0.60
PA EDV cm/s	7.75 ± 2.2	8.01 ± 2.1	0.56
PA TAV cm/s	16.18 ± 2.5	18.61 ± 4.9	0.004
PA PI	2.47 ± 0.36	2.08 ± 0.33	0.0001
PA RI	0.86 ± 0.05	0.78 ± 0.09	0.0001
PA TVI cm	5.3 ± 2.1	6.2 ± 1.7	0.026
PA AT ms	43 [40-47]	57 [47–60]	0.005
PA ET ms	177 [163-183]	187 [177-207]	0.005
PA AT:ET	0.25 [0.24–0.28]	0.32 [0.26– 0.34]	0.005

Abbreviations: PA, pulmonary artery; PSV, peak systolic velocity; EDV, end diastolic velocity; TAV, time-averaged velocity; PI, pulsatility index; RI, resistance index; TVI, time velocity integral; AT, acceleration time; AT:ET, acceleration time to ejection time ratio; cm, centimetres; cm/s, centimetres per second; ms, millisecond. MH, maternal hyperoxygenation. Values displayed as means ± SD or median and [IQR].

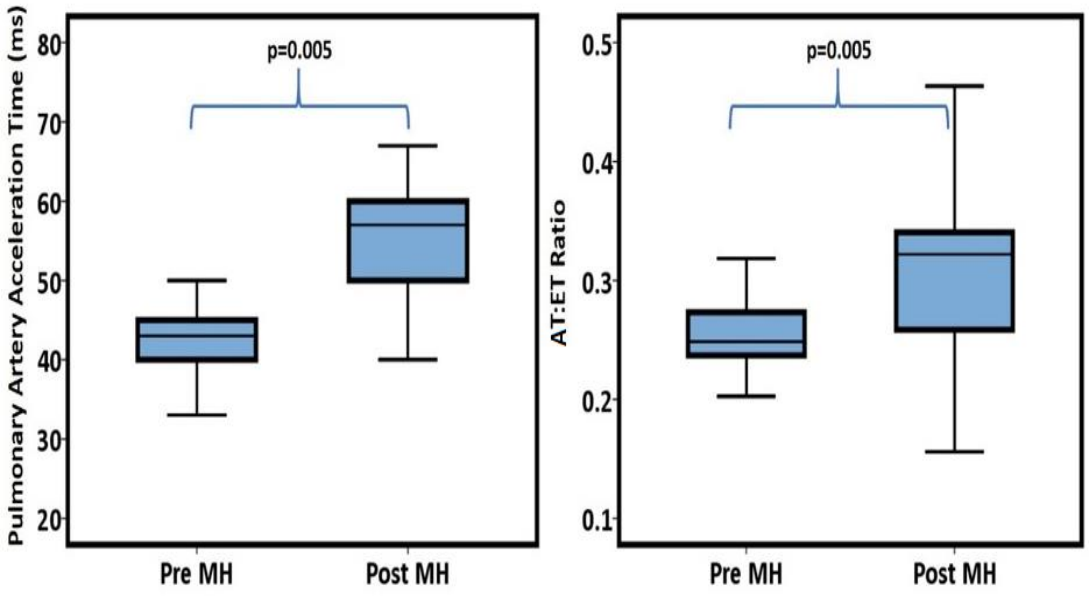
Figure 3.6 Pulmonary Artery Pulsatility Index Values Pre and Post Maternal Hyperoxygenation in each Individual Subject



Spaghetti plot illustrating actual pulmonary artery pulsatility index values in each pregnant subject pre and post MH.

Abbreviations: PI pulsatility index; Pre-MH; preceding maternal hyperoxygenation; Post-MH, following maternal hyperoxygenation.

Figure 3.7 Changes in Pulmonary Artery Acceleration to Ejection Time Ratio (AT:ET) following Maternal Hyperoxygenation



Abbreviations: Pre MH, before maternal hyperoxygenation; Post MH, following maternal hyperoxygenation; AT:ET, acceleration to ejection time ratio

Values displayed as medians and IQR

In 28 of the 46 participants (61%, n=28/46) the mean PA PI decreased by ≥10% from the baseline. This cohort were classified as responders. The response to MH based on participant group is described in *Table 3.4*. Of the 11 subjects recruited to group A, eight were responders (73%, n=8/11) and three were non-responders (27%, n=3/11). In group B, six subjects were responders (50%, n=6/12) and six were non-responders (50%, n=6/12). Of the three subjects recruited to group C, two subjects were responders (67%, n=2/3) and one was a non-responder (33%, n=1/3). Of the 20 subjects recruited to group D, 12 were responders (60%, n=12/20) and eight were non-responders (40%, n=8/20).

There was no difference in the response to MH when analysed per group (*Table 3.4*; Pearson Chi-square, p=0.38, likelihood ratio, p=0.29, linear-by-linear association, p=0.53). The PA PI response between responders and non-responders was not associated with maternal age (31.7 ± 6.4years vs. 33.8 ± 5.7years, p=0.29), maternal BMI (29.3 ± 4.8kg/m^2 vs. 31.2 ± 6.6kg/m^2, p=0.29) or GA at the time of MH (35.3 ± 2.2weeks vs. 34.9 ± 2.4weeks, p=0.57).

Table 3.4 Participant Groups and Prenatal Response to Maternal Hyperoxygenation

Group	Responders (n= 28)	Non-responders (n=18)	Total (n=46)
A	08 (73%)	03 (27%)	11
B	06 (50%)	06 (50%)	12
C	02 (67%)	01 (33%)	03
D	12 (60%)	08 (40%)	20

Group A: Fetuses at risk of pulmonary hypoplasia (mid-trimester PPROM, CDH, skeletal dysplasia and persistent oligohydramnios)

Group B: Women attending for scheduled CS prior to 38 weeks' GA

Group C: Fetuses with a prenatal diagnosis of moderate or severe perimembranous VSD or AVSD in the absence of other structural heart disease including fetuses with Trisomy 21.

Group D: A group of gestation-matched uncomplicated singleton pregnancies serving as a control group.

3.4.3 Aortic Isthmus Doppler

A satisfactory AoI Doppler waveform was obtained from 45 of the 46 participants (97.8%, n=45/46). All AoI Doppler waveform measurements were recorded in the LAA sonographic plane as previously described in methods *Section 2.3.6.* There was a decrease in mean fetal AoI PI following MH (2.61 ± 0.28 to 2.46 ± 0.36, p=0.04). Furthermore, the mean AoI RI decreased in response to MH (0.88 ± 0.05 to 0.86 ± 0.05, p=0.03) *(Table 3.5).* There were no changes in mean AoI PSV (95.1 ± 9.5cm/s vs. 94.3 ± 8.8cm/s, p=0.26), EDV (8.7 ± 3.1cm/s vs. 8.6 ± 4.2cm/s, p=0.89) or in the TAmax (33.8 ± 9.9cm/s vs. 35.7 ± 9.2cm/s, p=0.34) in the AoI following MH.

3.4.4 Ductus Arteriosus Doppler

Satisfactory DA Doppler waveforms were obtained in 45 of all 46 participants (97.8%, n=45/46). All DA Doppler waveforms were obtained in the LDA view as previously described in methods *Section 2.3.6*. The mean DA PSV, EDV and TAmax were all within normal reference ranges (338) for GA at 106.8 ± 15.4cm/s, 12.4 ± 1.8cm/s and 34.4 ± 7cm/s respectively. The mean DA PI did not change pre and post MH (2.41 ± 0.36 vs. 2.42 ± 0.35, p=0.89). The mean DA RI did not change pre and post MH (0.87 ± 0.10 vs. 0.88 ± 0.12, p=0.74) (*Table 3.5*). The measured values pre and post MH for the mean DA PSV (106.8 ± 15.4cm/s vs.109.1 ± 16.7cm/s, p=0.47), DA EDV (12.4 ± 1.8cm/s vs. 11.9 ± 2.2cm/s, p=0.24) and DA TAmax (34.4 ± 7.1cm/s vs. 36.4

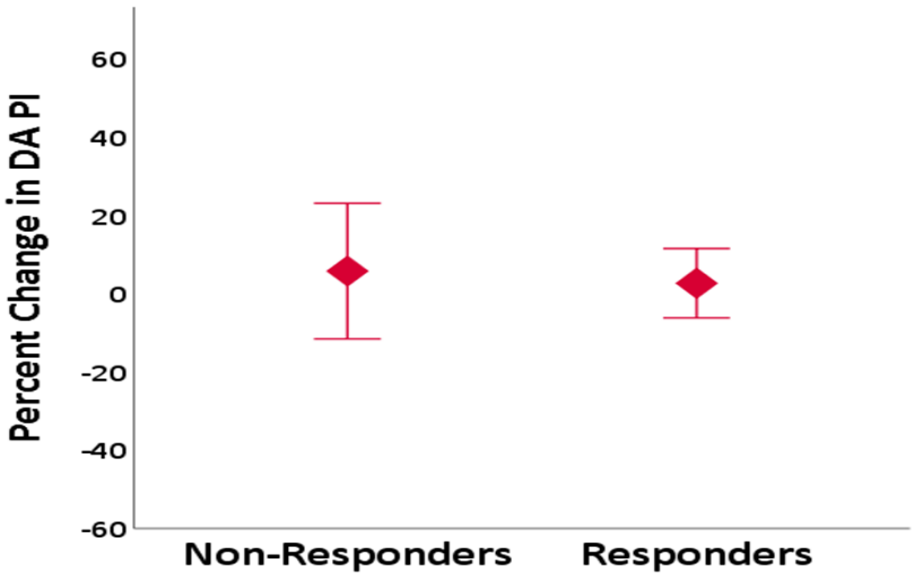
± 6.9cm/s, p=0.18) did not change significantly following MH *(Table 3.5).* The percentage change in the DA PI from the baseline was similar in those that responded to MH and in those that did not respond to MH *(Figure 3.8).*

Table 3.5 Changes in Aortic Isthmus and Ductus Arteriosus Doppler waveforms in response to Maternal Hyperoxygenation

Doppler measurements	**Pre MH**	**Post MH**	**p-value**
AoI PSV cm/s	95.1 ± 9.5	94.3 ± 8.8	0.26
AoI EDV cm/s	8.7 ± 3.1	8.6 ± 4.2	0.89
AoI TAmax cm/s	33.8 ± 9.9	35.7 ± 9.2	0.34
AoI PI	2.61 ± 0.28	2.46 ± 0.36	0.04
AoI RI	0.88 ± 0.05	0.86 ± 0.05	0.03
DA PSV cm/s	106.8 ± 15.4	109.1 ± 16.7	0.47
DA EDV cm/s	12.4 ± 1.8	11.9 ± 2.2	0.24
DA TAmax cm/s	34.4 ± 7.1	36.4 ± 6.9	0.18
DA PI	2.41 ± 0.36	2.42 ± 0.35	0.89
DA RI	0.87 ± 0.10	0.88 ± 0.12	0.74

Abbreviations: AoI, Aortic Isthmus; PA, pulmonary artery; DA, ductus arteriosus; PSV, peak systolic velocity; EDV, end diastolic velocity; TAmax, time-averaged maximum velocity; PI, pulsatility index; RI, resistance index. Values reported are means ± SD.

Figure 3.8 Percentage Change in the Ductus Arteriosus Pulsatility Index in Responders and Non-responders to Maternal Hyperoxygenation



This graph illustrates that the percentage (%) change in the DA PI from the baseline was similar in those that responded to MH and in those that did not respond to MH.

Abbreviations: DA, ductus arteriosus; PI, pulsatility index.

Figures shown as % (y axis)

Groups responders and non-responder's (x axis)

3.4.5 Umbilical Artery Doppler

A satisfactory UAD waveform was obtained in all 46 participants (100%, n=46/46). The mean UA PSV (39.8 ± 10cm/s vs. 41.6 ± 11cm/s, p=0.28), and EDV (16.1 ± 6cm/s vs. 16.2 ± 5cm/s, p=0.85) did not change in response to MH. No changes were observed in the mean resistance indices of the UA *(Table 3.6)* or in the TAmax (26.1 ± 7.5cm/s vs. 26.9 ± 7.1cm/s, p=0.60). The mean UA PI did not change following MH (0.96 ± 0.25 vs.0.99 ± 0.23, p=0.55). The mean UA RI did not change following MH (0.61 ± 0.09 vs. 0.62 ± 0.08, p=0.58). There was no change in the mean CPR pre and post MH (1.7 ± 0.5 vs. 1.7 ± 0.4, p= 1.00).

3.4.6 Middle Cerebral Artery Doppler

A satisfactory MCA Doppler waveform was obtained and analysed in all 46 participants (100%, n=46/46). There was a significant increase in mean MCA blood flow assessed using PSV pre and post MH (33.3 ± 11.5cm/s vs. 40.5 ± 17cm/s, p=0.02). There was no change in the mean MCA EDV (7.1 ± 3.2cm/s vs. 8.7 ± 5.3cm/s, p=0.08), PI (1.70 ± 0.6 vs. 1.72 ± 0.7, p=0.88) or RI (0.78 ± 0.09 vs. 0.80 ± 0.08, p=0.26) in response to MH *(Table 3.6).*

Table 3.6 Changes in Utero-placental Doppler Indices in Response to Maternal Hyperoxygenation in the Third Trimester

Doppler measurements	**Pre MH**	**Post MH**	**p-value**
UAD PSV cm/s	39.8 ± 10	41.6 ± 11	0.28
UAD EDV cm/s	16.1 ± 6	16.2 ± 5	0.85
UAD TAmax cm/s	26.1 ± 7.5	26.9 ± 7.1	0.60
UAD PI	0.96 ± 0.25	0.99 ± 0.23	0.55
UAD RI	0.61 ± 0.09	0.62 ± 0.08	0.58
MCA PSV cm/s	33.3 ± 11.5	40.5 ± 17	0.02
MCA EDV cm/s	7.1 ± 3.2	8.7 ± 5.3	0.08
MCA PI	1.70 ± 0.6	1.72 ± 0.7	0.88
MCA RI	0.78 ± 0.09	0.80 ± 0.08	0.26
CPR	1.7 ± 0.5	1.7 ± 0.4	1.00

Abbreviations: UAD, umbilical artery; MCA, middle cerebral artery; PSV, peak systolic velocity; EDV, end diastolic velocity; TAmax, time-averaged maximum velocity; PI, pulsatility index; RI, resistance index; CPR, Cerebroplacental ratio; cm/s, centimetres per second. Values reported are means ± SD.

3.4.7 Ductus Venosus Doppler

Good quality DV Doppler waveforms could not be obtained in each case, owing to fetal position, movements, or advanced GA. Satisfactory DV Doppler recordings were obtained from 59% (n=27/46) of participants. Qualitative assessment of the DV waveform confirmed a positive ‘a’ wave in all fetuses (100%, n=46/46). Ductus venosus mean systolic (60.2 ± 5.6cm/s to 65.2 ± 8.3cm/s, p=0.01), diastolic (52.1 ± 5.7cm/s to 55.8 ± 5.6cm/s, p=0.02) and ‘a’ wave (31.5 ± 6.4cm/s to 36.1 ± 6.6cm/s, p=0.01) velocities increased following MH. The DV Doppler ratios including PIV (0.84 ± 0.21 vs. 0.89 ± 0.21, p=0.39), PLI (0.58 ± 0.14 vs 0.54 ± 0.13, p=0.28) and PVIV (0.60 ± 0.11 vs. 0.57 ± 0.15, p=0.40) did not change in response to MH *(Table 3.9).*

Table 3.7 Changes in Ductus Venosus Doppler Measurements in Response to Maternal Hyperoxygenation

DV measurement n=27	Pre-MH	Post-MH	p-value
Systole cm/s	60.2 ± 5.6	65.2 ± 8.3	0.01
Diastole cm/s	52.1 ± 5.7	55.8 ± 5.6	0.02
A wave cm/s	31.5 ± 6.4	36.1 ± 6.6	0.01
PIV	0.84 ± 0.21	0.89 ± 0.21	0.39
PLI	0.58 ± 0.14	0.54 ± 0.13	0.28
PVIV	0.60 ± 0.11	0.57 ± 0.15	0.40

Abbreviations: Pre-MH, pre maternal hyperoxygenation; Post-MH, post maternal hyperoxygenation; PIV pulsatility index for veins; PLI, preload index; PVIV, peak velocity index for veins,

Values reported are means ± SD.

3.5 Reproducibility

Pulmonary artery Doppler waveforms were recorded from a subset of 10 patients. In each of the ten fetuses, the recording was made from the DPA in the sampling location described in *Section 2.3.3* at an interval of approximately 15 minutes. Both the Doppler recording and waveform analysis was performed by the same investigator (A.M.). Pregnancy duration ranged between 26 and 32 weeks in all cases. Intraobserver and interobserver variability of PA Doppler flow velocities (PI, RI) were low, with mean percent errors ranging from 4% to 6%. The intraobserver repeatability for PA PI and PA RI measurements showed high levels of ICC (0.99 and 0.97 respectively) and measures of CV of <10% (PA PI 9%, PA RI 4% (*Table 3.8*). Interobserver reproducibility of the PA PI (*Figure 3.10*) and PA RI showed high levels of ICC (0.98 and 0.93 respectively) (*Table 3.9*).

Table 3.8 Intraobserver Repeatability of Pulmonary Artery Doppler Measurements

Doppler Parameter	**Mean ± SD**	**MD**	**SDoD**	**RC**	**CV (%)**	**ICC (95% CI)**
PA PI (A.M) PA PI (F.B) R	2.33 ± 0.14 2.32 ± 0.12	0.015	0.046	0.28	9	0.99 (0.96-0.99)
PA RI (A.M) PA RI (F.B) R	0.87 ± 0.03 0.87 ± 0.04	0.024	0.012	0.59	4	0.97 (0.88-0.99)

Abbreviations: PA, pulmonary artery; PI, pulsatility index; RI, resistance index; MD mean difference of repeated tests; SD, standard deviation; SDoD, standard deviation of differences of repeated tests; RC, repeatability coefficient; CV, coefficient of variation, ICC, intraclass correlation coefficient.

A.M and F.B denote the study investigator that obtained the Doppler measurement.

R denotes the repeatability measurements.

Table 3.9 Interobserver Repeatability of Pulmonary Artery Doppler Measurements

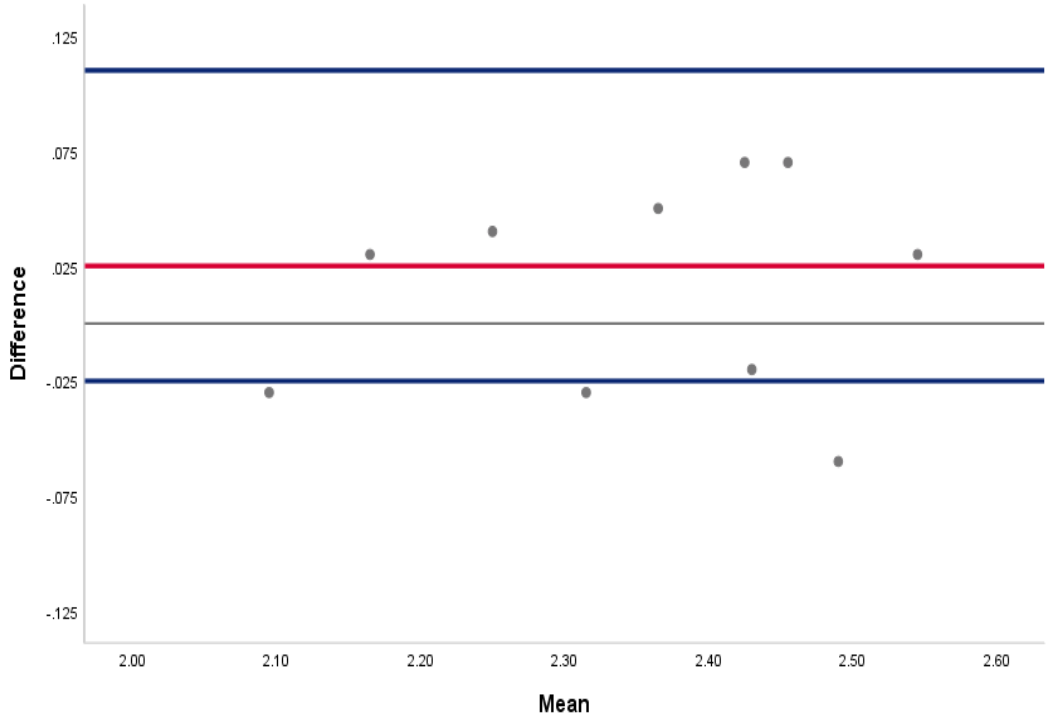
Doppler Parameter	**Mean ± SD**	**Difference (95% CI)**	**ICC (95% CI)**
PA PI (A.M) PA PI (F.B) R	2.35 ± 0.14 2.36 ± 0.15	0.046 (-0.075 - 0.11)	0.98 (0.90-0.99)
PA RI (A.M) PA RI (F.B) R	0.86 ± 0.03 0.86 ± 0.04	0.12 (-0.024-.024)	0.93 (0.71-0.98)

Abbreviations: PA, pulmonary artery; PI, pulsatility index; RI, resistance index; SD, standard deviation; SD of differences; standard deviation of repeated tests; ICC, intraclass correlation coefficient.

A.M and F.B denote the study investigator that obtained the Doppler measurement.

R denotes the repeatability measurements.

Figure 3.9 Interobserver Variation in Pulmonary Artery Pulsatility Index Measurements



Bland Altman plot showing the interobserver variation in PA PI measurements.

Mean PA PI (x axis). Difference in PA PI values between two independent operators (A.M and F.B).

Standard deviation of difference (Red line). Confidence intervals upper and lower limits (Blue line). Grey line represents zero.

3.6 Neonatal Outcomes

All babies were delivered in the Rotunda Hospital (100%, n=46/46). The mean GA at delivery was 37.5 ± 1.6 weeks. The mean gestational birthweight was 3212 ± 510g. A physical examination at birth was recorded as normal for all newborn subjects (100%, n=46/46). A neonatal echocardiogram was performed on 74% (n=34/46) of recruited cases. Neonatal echocardiography was carried out at a median age of 14 hours [12–24]. Of the neonates who underwent an echocardiogram, over half (56%, n=19/34) were previously classified as responders to MH and the remaining 44% (n=15/34) were previously classified as non-responders to MH. Of the 26% (n=12/46) of neonates that did not undergo an echocardiogram, one had a fragile skin condition at birth and the test was deferred, one declined participation and 10 infants were discharged home from the hospital before one of the study investigators could perform the neonatal echocardiogram. Of the 12 neonates (26%, n=12/46) that did not undergo an echocardiogram, nine (75%, n= 9/12) had been classified as responders to the MH test prenatally. Of all the neonates that underwent an echocardiogram, over half (53%, n=18/34) were delivered by CS. No neonate met criteria for a diagnosis of PPHN.

Neonates were divided into those that responded to MH in utero (Responders, 56%, n=19/34) and those that did not respond to MH in utero (Non-responders, 44%, n=15/34). There were no differences in mean GA at delivery (37.4 ± 1.9weeks vs. 38.0 ± 1.6weeks, p=0.32) or in mean

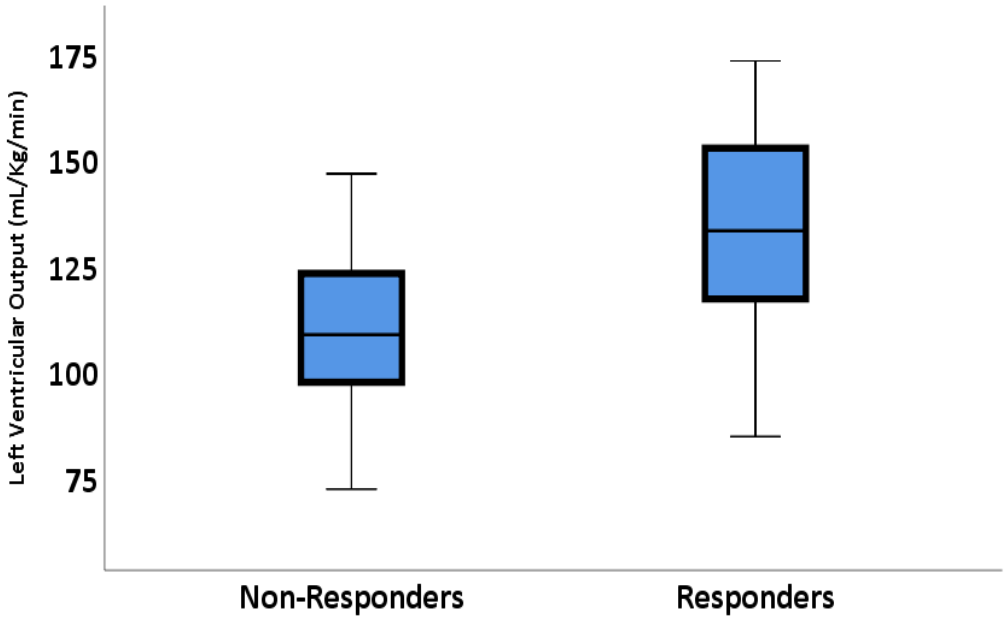
birthweight (3190 ± 611g vs. 3234 ± 409g, p=0.80) between responders and non-responders *(Table 3.10).* Fetuses that responded to MH (a decrease in the PA PI ≥10% from baseline) had a higher mean LVO (135 ± 25 mL/Kg/min vs. 111 ± 21 mL/Kg/min, p=<0.01) *(Figure 3.11)* and EF (54 ± 9% vs. 47 ± 7%, p=0.03) within the first 24 hours of life when compared to non-responders (*Figure 3.12*). These findings were not dependent on mean LV length (28 ± 4mm vs. 28 ± 4mm, p=0.88) or mean mitral valve annular diameter (8.6 ± 1.4mm vs. 9.4 ± 0.9mm, p=0.08) but were related to an increase in mean mitral valve inflow (8.6 ± 1.6cm vs. 7.4 ± 0.9cm, p=0.01) in responders when compared with non-responders. There were no differences in mean nPAAT (78.6 ± 26 vs. 78.3 ± 21, p=0.53), mean RV end systolic pressure (18.6 ± 12.2mmHg vs. 17.4 ± 6.9mmHg, p=0.81) or mean PDA diameter (2.6 ± 1.3mm vs 2.4 ± 1.5mm, p=0.74) between the two groups.

Table 3.7 Neonatal Echocardiography Data

	Responders (n=19)	**Non-responders (n=15)**	**p-value**
Gestation (weeks)	37.4 ± 1.9	38.0 ± 1.6	0.32
Birthweight (grams)	3190 ± 611	3234 ± 409	0.80
LV length (mm)	28 ± 4	28 ± 4	0.88
Mitral Valve Annular diameter (mm)	8.6 ± 1.4	9.4 ± 0.9	0.08
Ejection Fraction (%)	54 ± 9	47 ± 7	0.03
Left Ventricular Output (mL/Kg/min)	135 ± 25	111 ± 21	<0.01
Mitral Valve Inflow VTI	8.6 ± 1.6	7.4 ± 0.9	0.01
nPAAT msec	78.6 ± 26	78.3 ± 21	0.53
RV end systolic pressure (mmHg)	18.6 ± 12.2	17.4 ± 6.9	0.81
PDA characteristics (mm)	2.6 ± 1.3	2.4 ± 1.5	0.74

Abbreviations: LV, left ventricle; VTI, velocity time integral, nPAAT, neonatal pulmonary artery acceleration time; RV, right ventricle, PDA, patent ductus arteriosus. All values reported are means ± SD.

Figure 3.10 Neonatal Left Ventricular Output in Responders and Non-responders to Maternal Hyperoxygenation

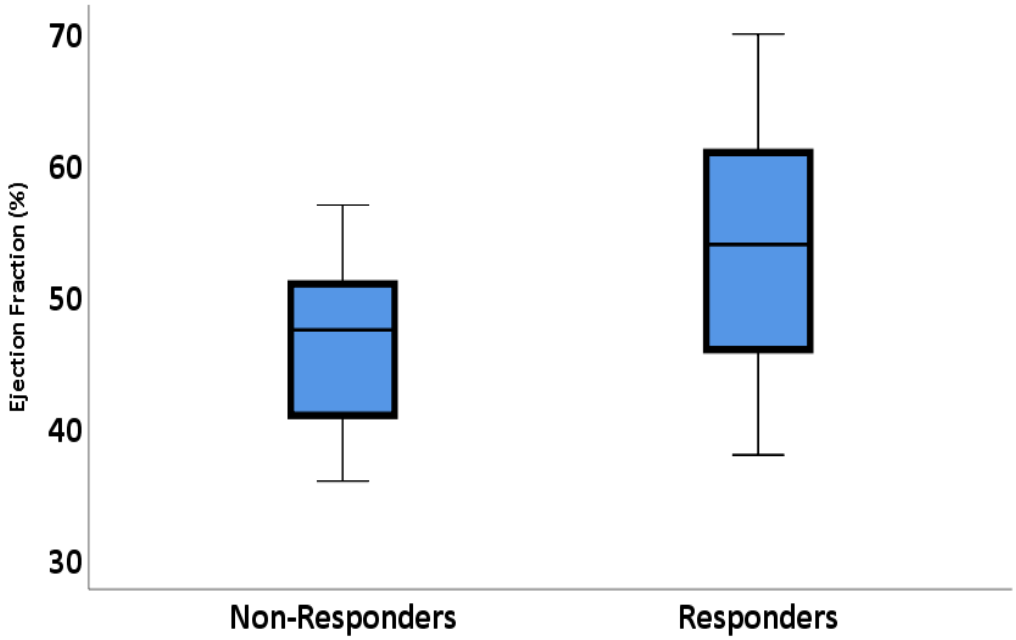


Displayed are the neonates who underwent a neonatal echocardiogram (n=34)

Neonates that responded (Responders, n=19) to maternal hyperoxygenation (x-axis). Neonates that did not respond (Non-responders, n=15) (x-axis).

Left ventricular output measurements (y axis) displayed in mL/kg/min.

Figure 3.11 Neonatal Ejection Fraction in Responders and Non-responders to Maternal Hyperoxygenation



Displayed are the neonates who underwent a neonatal echocardiogram (n=34).

Neonates that responded (Responders, n=19) to maternal hyperoxygenation (x-axis). Neonates that did not respond (Non-responders, n=15) (x-axis).

Ejection fraction as measured by echocardiogram (y axis) displayed as %.

The effect of MH on neonatal LVO was not dependent on mode of delivery (standardised β=0.13, p=0.48), GA at delivery (standardised β=0.05, p=0.80) or infant HR (standardised β=-0.05, p=0.77) at the time of the neonatal echocardiogram. However, neonatal LVO was dependent on the prenatal response to MH (standardised β=0.51, p<0.01).

The neonatal EF was also not dependent on the mode of delivery (standardised β=-0.17, p=0.29) or GA at delivery (standardised β=0.02, p= 0.89) but was dependent on the prenatal response to MH (standardised β=0.34, p=0.04) and on infant HR (standardised β=-0.41, p=0.02), *(Table 3.11).*

Table 3.8 The Independent Effect of the response to Maternal Hyperoxygenation on Neonatal Left Ventricular Output (Model 1) and Ejection Fraction (Model 2)

Dependent Variable	**Model 1: LVO**		**Model 2: EF**	
Predictor Variables	Standardised β	p	Standardised β	p
MH Response	0.51	<0.01	0.34	0.04
Mode of Delivery	0.13	0.48	-0.17	0.29
Gestation at Delivery	0.05	0.80	0.02	0.89
Infant Heart Rate	-0.05	0.77	-0.41	0.02

Linear Regression Analysis assessing the independent effect of maternal hyperoxygenation response on neonatal left ventricular output (Model 1) and ejection fraction (Model 2).

Abbreviations: LVO, left ventricular output; EF, ejection fraction; MH, maternal hyperoxygenation.

Seven neonates (15%, n=7/46) were admitted to the NICU post-delivery. Reasons for NICU admissions are outlined in *Table 3.12.* Two neonates (4.3%, n=2/46) were admitted to the NICU with TTN. Of those, one neonate (2.2%, n=1/46) required ventilation for four hours following delivery at 37 weeks GA by elective CS. The CS was performed for a maternal diagnosis of placenta praevia. Notably that neonate was a non-responder to MH in utero. The second neonate admitted to the NICU with TTN was born at 33+1 weeks GA by a vaginal breech delivery following a diagnosis of PPROM at 31+5 weeks gestation. Notably that neonate was a responder to MH in utero and decreased its PA PI by 16% following MH. This neonate had a protracted stay in the NICU (seven weeks) due to the development of necrotising enterocolitis (NEC) complicated by a bowel perforation requiring a bowel resection with an end-to end anastomosis.

Table 3.9 Indication for Admission to the Neonatal Intensive Care Unit in all HOTPOT Study Participants

Indication for Admission	**Numbers (%) n=7**
Transient Tachypnoea of the Newborn	2 (28.6%)
Hypoglycaemia	2 (28.6%)
Jaundice	1 (14.3%)
Dermatological condition	1 (14.3%)
Heart rate monitoring	1 (14.3%)

Admission to the NICU occurred in 15% (n=7/46) of all study participants. Indications for NICU admission are displayed above. Values are displayed as absolute numbers and percentages.

3.6.1 Neonatal Response per Group

Group A: *Fetuses at risk of pulmonary hypoplasia (mid-trimester PPROM, CDH, skeletal dysplasia, and persistent oligohydramnios).*

Of the 11 fetuses recruited to the study, eight (73%, n=8/11) responded to MH and three (27%, n=3/11) did not. Of those that responded, six (75%, n=6/8) had a neonatal echocardiogram and two (25%, n=2/8) did not. Of those that did not respond prenatally (27%, n=3/11), two neonates (67%, n=3/3) had a neonatal echocardiogram and one (33%, n=1/3) did not (*Table 3.13*).

Group B: *Women attending for scheduled CS prior to 38 weeks' GA.*

Of the 12 fetuses recruited to group B, six (50%, n=6/12) responded to MH in utero and six (50%, n=6/12) did not. Of the six fetuses that responded to MH, three (50%, n=3/6) had a neonatal echocardiogram and three (50%, n=3/6) did not. Of the six fetuses that did not respond to MH prenatally, all six (100%, n=6/6) underwent a neonatal echocardiogram (Table 3.13).

Group C: *Fetuses with a prenatal diagnosis of a moderate or severe perimembranous VSD or AVSD in the absence of other structural heart disease including fetuses with Trisomy 21.*

Three fetuses were recruited to this group. Two (67%, n=2/3) responded to MH in utero and one (33%, n=1/3) did not. Of the two fetuses that responded to MH, both neonates (100%, n=2/2) underwent a neonatal echocardiogram in the postnatal period and both had no VSD documented at the time of the neonatal echocardiogram. The third participant in this group responded to MH in utero however, this neonate was transferred from our hospital to another hospital prior to completion of the neonatal echocardiogram and was therefore lost to follow-up (Table 3.13).

Group D: *A group of gestation-matched uncomplicated singleton pregnancies serving as a control group.*

Of the 20 fetuses recruited to this group, 12 (60%, n=12/20) responded to MH in utero and eight (40%, n=8/20) did not. Of those that responded, eight (67%, n=8/12) underwent a neonatal echocardiogram and four (33%, n=4/12) did not. Of the eight fetuses that did not respond to MH prenatally, seven (88%, n=7/8) underwent an echocardiogram in the neonatal period and one (12%, n=1/8) did not (Table 3.13).

Table 3.10 Neonatal Echocardiograms Performed by Recruitment Group

Group	***Fetal Response***	**Neonatal Echocardiogram in Responder group**	***No fetal response***	**Neonatal Echocardiogram in Non-responder group**
A (n=11)	*8 (73%)*	6 (75%)	*3 (27%)*	2 (67%)
B (n=12)	*6 (50%)*	3 (50%)	*6 (50%)*	6 (100%)
C (n=03)	*2 (67%)*	2 (100%)	*1 (33%)*	0 (0%)
D (n=20)	*12 (60%)*	8 (67%)	*8 (40%)*	7 (88%)

Group A: Fetuses at risk of pulmonary hypoplasia: mid-trimester PPROM, CDH, skeletal dysplasia, and persistent oligohydramnios. Group B: Women attending for scheduled CS prior to 38 weeks' GA. Group C: Fetuses with a prenatal diagnosis of moderate/ severe perimembranous VSD/AVSD in the absence of other structural heart disease including fetuses with Trisomy 21. Group D: A group of gestation-matched uncomplicated singleton pregnancies serving as a control group.

Values displayed as absolute numbers and %.

3.7 Paediatric Follow-up

Of all pregnant participants, 44 out of 46 (96%, n=44/46) completed the scheduled follow-up. Outpatient paediatric follow-up was performed in 11 cases (25%, n=11/44). The majority of participant follow-up was performed by telephone (75%, n=33/44). The mean length of time to follow-up was 45.4 days (range 42-70 days). No neonate had a diagnosis of PPHN, RDS, periventricular leukomalacia (PVL), hypoxic ischaemic encephalopathy (HIE) or talipes at 6-week follow-up. One neonate had a dermatological diagnosis of lamellar icthyosis. One neonate had a diagnosis of NEC. Details of that neonate's postnatal course were outlined in *Section 3.7.* Two participants (4.3%, n=2/46) were lost to paediatric follow-up.

3.8 Feasibility, Acceptability, Safety

3.8.1 Feasibility

The proportion of eligible women that were approached who agreed to participate in the study

- There were lower than anticipated numbers of recruits in groups A and C of the study over the study period. The majority (83%, n=5/6) of patients with mid-trimester PPROM who had initially consented to partake in the study, delivered prior to 31 weeks gestation, and therefore did not meet criteria for inclusion.

- All non-pregnant controls that were approached to participate in the study participated (100%, n=20/20).

The proportion of women in whom it was possible to obtain PA Doppler velocimetry measurements

- It was possible to obtain PA Doppler waveforms for all pregnant participants (100%).
- Ease of acquisition for all Doppler waveforms in the study is represented in *Table 3.12*.
- Obtaining the PA Doppler waveform was rated as ‘very easy’ or ‘easy’ in 71% (n=33/46) of cases. It was difficult to obtain a PA Doppler waveform in 7% (n=3/46) of cases.

Satisfactory collection of all endpoints and variables

- All endpoints and variables were recorded in the CRF (100%). All variables were data source verified by the data safety monitor (Ms Mandy Jackson) in 50% (n=23/46) of all pregnant participants and 100% (n=20/20) of non-pregnant participants at the DSM meetings *(Section 2.6).*
- A neonatal echocardiogram was performed on 74% (n=34/46) of recruited cases. In total, 12 neonates (26%, n=12/46) did not

undergo a neonatal echocardiogram, one had a fragile skin condition at birth and the test was deferred, one was transferred out to another hospital and 10 infants were discharged home from the hospital before one of the study investigators could perform the echocardiogram.

- One neonate (2%, n=1/46) was transferred to a different hospital before the neonatal echocardiogram could be completed and was subsequently lost to follow-up. One further paediatric follow-up was not completed due to an inability to contact the participant.

Economic considerations

- A NICOM® monitor costs €6,500. Once purchased it can be used repeatedly to obtain haemodynamic variables. The single use sensors (one pack of 4 electrodes) cost €100. There was a NICOM® machine in our department which had been previously purchased and this was used for the study. The original budget details and justification, for the study can be found in the *Appendices*. The costs in performing a repeat study of the same size are described in *Table 3.15.*

Table 3.11 Ease of Doppler Acquisition Pre and Post Maternal Hyperoxygenation in a Single Operator

	Pulmonary Artery	**Ductus Arteriosus**	**Aortic Isthmus**	**Ductus Venosus**	**Umbilical Artery**	**Middle Cerebral Artery**
Obtained	***46 (100%)***	***45 (98%)***	***45 (98%)***	***27 (59%)***	***46 (100%)***	***46 (100%)***
Very easy	14 (30%)	7 (15%)	6 (13%)	4 (9%)	45 (98%)	15 (33%)
Easy	19 (41%)	15 (33%)	14 (30%)	10 (22%)	1 (2%)	24 (52%)
Fair	10 (22%)	18 (39%)	20 (44%)	9 (20%)	0 (0%)	4 (9%)
Difficult	3 (7%)	5 (11%)	5 (11%)	4 (9%)	0 (0%)	3 (6%)
Unobtainable	0 (0%)	1 (2%)	1 (2%)	19 (41%)	0 (0%)	0 (0%)

Ease of Doppler acquisition rated by a single operator (A.M) at the completion of the ultrasound study visit (ratings include the overall ease of acquisition of both Doppler waveforms, i.e. the first pre hyperoxygenation and the second, following hyperoxygenation).

Ease of acquisition measurements adapted from Mone et al (344).

Table 3.12 Cost to Perform a Study of a Similar Size

Resource/Consumables	**Cost**
NICOM® machine	€6,500
NICOM® stickers €100 per packet (4 stickers) 1 box = 25 packets (2,500e) Require 3 boxes	€7,500
Oxygen cylinder (compressed gas) Given at 10-12Litres/minute x 10minutes 100 litres x 66 patients Total: 6,600 Litres of Oxygen Small cylinder €9.46 with connector	€20
Oxygen face mask (non-rebreather type) €1 per mask 1 box (24 masks) Require 3 boxes	€75
All Consumables	TOTAL
Variable costs Fixed costs (NICOM® machine) Total	€ 7,595 € 6,500 €14,095

** Depreciation of NICOM® machine not included*

3.8.2 Acceptability

Adherence to study procedures

Fetal ultrasound

- All pregnant participants completed the ultrasound aspect of the study (100%, n=46/46).
- The mean length of time for completion of the study visit was 72 ± 15 minutes for the pregnant participants.

NICOM® monitoring

- All participants completed the NICOM® monitoring (100%, n=66/66).
- In three cases (4.5%, n=3/66) the monitor reported an error in receiving the signal from the NICOM® stickers, these needed to be replaced and an additional set of stickers were used.

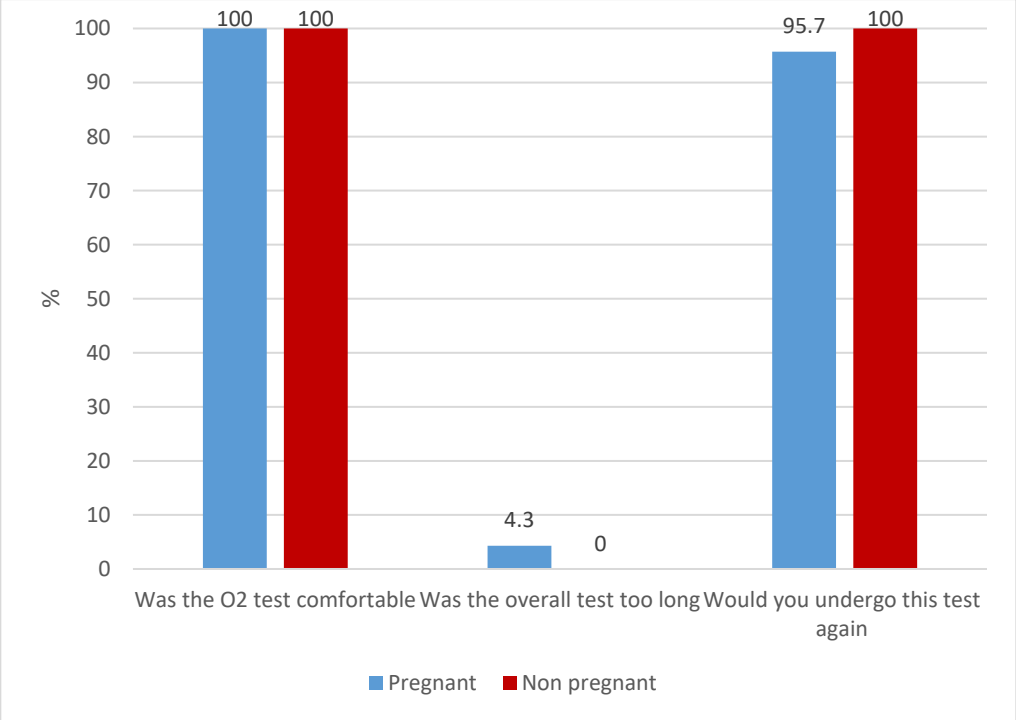
Oxygen administration

- The acceptability of oxygen administration was evaluated by a questionnaire *(Figure 3.12).*
- All participants answered the questionnaire (100%). All participants (100%, n=66/66) described the hyperoxygenation test as being comfortable. Two pregnant

participants (4.3%, n=2/46) reported that the overall test was too long. The same two participants reported that they would not undergo the test again.

- No participant reported any side effects during or after the administration of hyperoxygenation (0%).

Figure 3.12 Hyperoxygenation Test Acceptability Questionnaire



Results of a questionnaire comprising of three questions:

Q1- Was the oxygen test comfortable?

Q2- Was the overall test too long?

Q3- Would you undergo this test again?

Results (percentages; y axis) displayed as response per group (pregnant vs non-pregnant; x axis).

3.8.3 Safety

Monitoring of maternal haemodynamics

- Maternal haemodynamics were monitored using the NICOM® system. We identified a potential safety point in relation to the administration of high flow oxygen to pregnant women.
- We demonstrated that hyperoxygenation in pregnancy exerts a more profound effect on cardiovascular haemodynamics than is observed in the non-pregnant state.
- In the pregnant group, there was a fall in mean CO (6.3 ± 1.1L/min to 6.1 ± 1.0L/min to 5.7 ± 1.0L/min, p=0.008) and CI (3.3 ± 0.5 L/min/m^2 to 3.2 ± 0.6 L/min/m^2 to 3.0 ± 0.5L/min/m^2, p=0.005) over the course of hyperoxygenation (baseline/ten minutes of MH/ten minutes post cessation of MH), coupled with a rise in mean SVR (1236 ± 286 dynes/sec/cm^{-5} to 1401 ± 301 dynes/sec/cm^{-5}, p=0.009).
- In the non-pregnant group, there was no significant change in the mean CI (2.8 ± 0.6L/min/m^2 vs. 2.7 ± 0.6L/min/m^2, p=0.60) or mean SVR (1509 ± 312dynes/sec/cm^{-5} vs. 1560 ± 427dynes/sec/cm^{-5}, p= 0.67) in response to hyperoxygenation.
- This work has received interest at a national and international level given the widespread use of oxygen in daily clinical obstetrics. This work was published in the American Journal of Obstetrics and Gynaecology in April 2019 *(Appendices).*

4 Chapter 4 DISCUSSION

4.1 Key findings

In this chapter, I provide a brief description of the results of each section followed by a discussion of the significance of these results and their context within the current literature. This thesis has increased our understanding of the complex pathway involved in the transition between fetal and neonatal life. This study was designed as a feasibility study and used a convenience sample over a given time interval (17 months), given no similar previous studies with control data. This study provides effect estimates on the response to MH in the third trimester and on neonatal echocardiographic indices.

We have demonstrated that MH leads to a decrease in PVR in fetuses in the third trimester, and that MH is feasible and acceptable in pregnancy. The reduction in PVR was demonstrated by a reduction in fetal PA PI and PA RI indices as well as by an increase in PA AT:ET in response to MH.

We have described improved neonatal echocardiographic indices of LVCO and EF in those that responded to MH in utero when compared to those that did not, suggesting that the prenatal response to MH may serve as a predictor of an optimal neonatal cardiac transition in the early newborn period.

We have observed that hyperoxygenation in pregnancy exerts a more profound effect on cardiovascular haemodynamics than is observed in the non-pregnant state and this raises concerns for the safety of injudicious oxygen use in pregnancy. This finding has gained interest at an international level since our discovery of this unique maternal response to hyperoxygenation.

4.2 Haemodynamic Changes

Despite the accepted uses for MH, the impact of hyperoxia on maternal haemodynamic indices has not been evaluated (123). As a result, there is a paucity of data in the literature in relation to the physiological changes to the maternal circulation in response to supplemental oxygen.

We have demonstrated that MH during the third trimester is associated with significant changes in maternal haemodynamic indices, characterised by a fall in CI and a rise in SVR, without recovery to baseline levels at ten minutes following cessation of MH. In our study, oxygen administration led to an increase in SVR which coincided with an acute reduction in resting HR and CI. The observed changes in HR were rapidly reversed after returning to room air concentrations. The decrease in CI and SV continued beyond the cessation of MH. The observed increase in SVR also persisted. Cardiac index and SVR returned to baseline levels at 30 minutes following the

cessation of hyperoxygenation in a subset of 22 pregnant participants who underwent NICOM® monitoring for an additional 20 minutes.

There was no baseline difference demonstrated in SV between the two groups, likely due to the fact that SV declines towards term and all pregnant patients in this study were greater than 31 weeks GA. An increased HR is maintained in the third trimester thus maintaining the increase in CO in the pregnant group at baseline.

During pregnancy, there is a significant increase in the demand for oxygen, owing to the increased metabolic rate and a 20% increased consumption of oxygen. Resting minute ventilation and tidal volume increase as does arterial pO_2 (345). A mild fully compensated respiratory alkalosis is normal in pregnancy (189). Simchem et al. have shown that 100% oxygen administration to pregnant women is associated with significant hypocapnia and hyperventilation. End tidal CO_2 (ET-CO_2) levels decreased by 12% during hyperoxygenation with 100% oxygen at a rate of 5L/min in the third trimester of pregnancy (108). Other studies that have explored the maternal response to hyperoxygenation have concluded that they did not observe any adverse maternal side effects or maternal complications during or after hyperoxygenation (169, 286). However, these studies did not objectively monitor maternal haemodynamic indices such as CO, SV or SVR. Our study recorded the haemodynamic response following a ten minute exposure to hyperoxygenation. This time interval was chosen as we were primarily

studying the fetal response and many studies assessing the fetal response to hyperoxia use a ten minute duration of oxygen exposure (110, 346, 347).

In non-pregnant literature, haemodynamic changes have been observed following a short duration of hyperoxygenation (348, 349). The haemodynamic effects of hyperoxygenation have been shown to occur in a dose-dependent fashion and can be detectable at inspired oxygen concentrations of 40% (350). A prospective study of 35 subjects evaluated the effects of hyperoxygenation on patients with congestive heart failure (CHF) versus those with normal LV function. Oxygen was administered as 21% O_2 by a non-rebreather mask, followed by 100% O_2 for 20 minutes, followed by 21% O_2 for a ten-minute recovery period. The authors concluded that hyperoxygenation was associated with impairment of LV relaxation and an increase in LV filling pressures (LVEDP) in patients with and without CHF, as measured by cardiac catheterisation (348). In a study of 27 patients undergoing elective cardiac catheterisation, hyperoxygenation was associated with an approximately 40% increase in coronary resistance and a 30% decrease in coronary blood flow (CBF) in the absence of any significant change in the diameter of large-conductance coronary vessels (349). The authors concluded that hyperoxygenation elicits vasoconstriction in the coronary circulation, functioning at the level of microvascular resistance vessels. These studies indicate that caution should be used when administering high inspired oxygen fractions to all normoxic patients.

Our study demonstrates that MH leads to an increase in cardiac parasympathetic activity, consistent with observations reported in previous studies of healthy non-pregnant volunteers (351, 352). The reduction in HR, therefore, is likely to reflect increased vagal activity. Parasympathetic activation appears to be unrelated to changes in BP, because there was no significant change in BP during hyperoxygenation in either group. The mechanism by which oxygen administration leads to a reduction in CO and an increase in SVR is poorly understood. One possible hypothesis is that hyperoxia leads to the generation of ROS (353), which in turn decreases the bioavailability of local NO (354) impairing NO dependent vasodilatation and increasing basal vascular tone (355). Under hyperoxic conditions ROS have been generated in vivo (353) and have been shown to readily degrade NO in vitro (356). Increased concentrations of ROS are known to have negative effects on myocardial function (357-359), and can impair myocyte Ca^{2+} homeostasis (348, 360, 361). Another plausible mechanism is that hyperoxia can induce vasoconstriction by acting directly on long lasting (L-type) Ca^{2+} channels (362). It has been shown in animal studies that oxygen sensitive L-type Ca^{2+} channels are present on vascular smooth muscle and are involved in the local circulatory control during hyperoxia (363).

In healthy non-pregnant subjects, the effect of acute oxygen administration has been examined in a series of studies using validated, non-invasive techniques (355, 364, 365). In fact, the effects of hyperoxia on cardiovascular function in non-pregnant subjects has been extensively

investigated. Some studies have demonstrated a reduction in HR and CI as well as a reduction in cardiac oxygen consumption and CBF (366-368) in response to hyperoxia. Others have highlighted an increase in BP and SVR and a decrease in CO in response to hyperoxia in healthy subjects (369). Studies in patients undergoing cardiac surgery have shown that hyperoxia results in a decrease in CI and an increase in SVR in those with a higher oxygen target during operative bypass when compared to those with a lower oxygen target (370, 371). However, there remains inconsistent data in the literature in relation to the effects of hyperoxygenation in different patient populations and there exists significant heterogeneity in the methodology particularly in the method of haemodynamic monitoring employed. A unique aspect of this study is the insight offered by NICOM® technology for both the pregnant and non-pregnant groups.

Accurate monitoring of haemodynamic outputs has traditionally been performed using invasive methods such as pulmonary artery catheterisation. It has also been performed by minimally invasive methods such as an arterial catheter for pulse contour analysis, intra-tracheal tube for partial CO_2 rebreathing, or continuous Doppler velocity flow assessment via a supra-sternal transthoracic ultrasound beam or oesophageal probe (372, 373). Methods to monitor maternal haemodynamics non-invasively have been evaluated and have gained recent interest (215-217).

Thoracic bioimpedance was the first non-invasive method used for CO monitoring (218). However, it has been found to be inaccurate in many

clinical settings, where significant electrical noise and body motion exist (374, 375). While bioimpedance-based systems rely on measured changes in signal amplitude, bioreactance-based systems such as NICOM® are based on an analysis of relative phase shifts of an oscillating current that occur when current traverses the thoracic cavity and are therefore less susceptible to noise (218).

In contrast to bioimpedance based systems, bioreactance does not depend on the distance between the electrodes when measuring CO and SV, which significantly reduces the uncertainty in the measured result (218). The NICOM® bioreactance system has been validated in the adult population by correlation with thermodilution (212). Following approval by the American Food and Drug Administration (FDA) in 2008, the NICOM® system has demonstrated acceptable accuracy, precision and responsiveness for CO monitoring in patients in a wide range of circulatory situations (212). The NICOM® system has shown high correlation with thermodilution in the ICU setting (211). It also demonstrates comparable CO and SV monitoring capabilities and pulse contour analysis calibrated by transpulmonary thermodilution (376). The NICOM® system has proven to be precise and reliable for obtaining measures of CO at rest and following a vasodilator challenge in patients with pulmonary hypertension (377). It can provide an accurate assessment of volume responsiveness in critically ill patients (378). Additionally, the NICOM® system has been shown to be a viable alternative

to guide goal-directed fluid therapy in colorectal surgical patients when compared to the oesophageal Doppler monitor (379).

Recent studies have demonstrated good agreement between NICOM® and 2D transthoracic echocardiography in estimating CO and SV, specifically in the third trimester of pregnancy (222). The NICOM® system has recently been validated against transthoracic echocardiography in the obstetric population (219). Measurements derived from the bioreactance-based NICOM® system correlate well with results derived from PA catheterisation (380, 381). Additionally, the NICOM® system has the advantage of being entirely operator independent and therefore not subject to any interobserver variation.

Maternal hyperoxygenation is commonly undertaken in obstetric practice. Many of the therapeutic and diagnostic practices using hyperoxia employ a rate of oxygen delivery ranging from 5-12L/min (108, 112, 133, 169, 171, 175, 346, 347). Despite the rate of oxygen delivery and although it may not be physiologically possible for fetal pO_2 to exceed maternal venous pO_2, MH clearly raises markers of free radical activity in both maternal and fetal blood (382-384). The generation of ROS in response to hyperoxia can lead to lipid peroxidation and tissue damage (385). Maternal hyperoxygenation is employed during CS under regional anaesthesia in many centres worldwide and reports on its safety are conflicting. Oxygen inhalation of 60%–100% during elective CS has been shown to increase arterial oxygen and oxygen free radicals in both the mother and fetus (382, 386). It has been suggested

that administering supplementary oxygen to healthy women during elective CS under spinal anaesthesia is unnecessary (387). One study identified increased indices of fetal oxygenation (umbilical arterial and vein PO_2 and oxygen content), following MH with 60% oxygen in patients undergoing non-elective CS under regional anaesthesia, without an increase in free radical activity (385) .

In animal studies, maternal oxygen supplementation increased fetal oxygenation but led to a concomitant increase in lipid peroxidation in fetal goats (384). In human studies, a higher incidence of fetal acidosis has been noted in the fetuses of women exposed to supplementary oxygen for more than ten minutes during vaginal delivery (388). In addition, a recent study concluded that among patients with a Category II FHR tracing in active labour, intrauterine resuscitation with room air was not inferior to oxygen in improving UA lactate (184). A Category II FHR tracing is defined as all FHR patterns on a cardiotocograph (CTG) that are not classified as category I (normal) or category III (abnormal) (389). The findings of this study are consistent with other trials that have failed to demonstrate a benefit on hypoxia-associated neonatal morbidity by administering supplemental oxygen to laboring women, suggesting that this recommended intervention may be a form of unnecessary treatment (118, 124, 388, 390, 391).

A Cochrane review in 2012 of two small studies including 246 women concluded that there is not enough evidence to support the use of prophylactic oxygen therapy for women in labour, nor to evaluate its

effectiveness for fetal distress (113). Notwithstanding this evidence, there is now data linking hyperoxygenation in infants with increased respiratory and neurological morbidity, which has prompted the American Academy of Pediatrics to review its recommendations on neonatal resuscitation and oxygen administration (184, 392). The potential negative effects of free radical damage on neonates is concerning, particularly for those born preterm with a decreased capability to counter the effects of free radical activity (393, 394). Our findings highlight the potential adverse cardiovascular effects of MH use in pregnancy. Further investigation into the benefits and risks of hyperoxygenation in an obstetric population is urgently required.

4.3 Pulmonary Artery Reactivity

We have demonstrated that MH during the third trimester is associated with significant changes in fetal PA Doppler waveforms, characterised by a decrease in the PA PI and PA RI and an increase in the PA AT:ET. These changes did not occur at the expense of ductal constriction. These findings were not related to a change in the resistance indices of the utero-placental circulation. Fetuses that were classified as responders to MH were more likely to have a higher LVO and EF during the early neonatal period. The neonatal echocardiographic changes are likely reflecting an increased capacity in those fetuses to increase pulmonary blood flow as a result of

decreased pulmonary resistance. The increased pulmonary venous return subsequently increased the left ventricular preload. This was reflected in both an increase in the left ventricular EF and in LVCO.

In the human fetus, blood flow velocity waveforms can be recorded from the right and left PAs or from peripheral vessels within the lung. Analysis of the waveforms using ultrasound Doppler can be used to study the normal development of the fetal lung circulation (395). Doppler examination of fetal PA blood flow is technically feasible, and it increases our understanding of fetal lung physiology. The walls of most fetal peripheral arteries including the pulmonary arteries increase their muscular component in the late third trimester (58). This enables a maximal response to various vasoconstrictive agents including oxygen and prostaglandin (396). The definition of three distinct segments of the pulmonary vasculature has been described (224). The distal and proximal PA yield the same information in relation to the pulmonary vascular reactivity since both demonstrate similar decreases in PI values during MH (54). The distal PA (beyond the first bifurcation of the branch PA) was chosen for our study, in keeping with Rasanen et al (54) and supported by Sivan et al (224) as a suitable area to consistently record PA Doppler indices.

Mean decreases in PA PI following MH of between 18.0 and 21.2% have been previously described in normal fetuses (54). A cut off level of a ≥20% decrease in the PA PI from the baseline has been studied and deemed to demonstrate pulmonary reactivity (54, 109). However, there remains large

individual variability (147, 223). This variability can also be identified when the same fetus is serially assessed as gestation advances (133). In one study of normal fetuses, nearly one-third of fetuses demonstrated a decrease in PA PI that was less than 20% after an initial positive oxygenation test earlier in gestation (133). Values for reactivity in our study accounted for this variability and were based on a previous study where a decrease in the PA PI of ≥10% from the baseline level was used to characterise a reactive test or positive responder (110).

The increase in fetal pulmonary blood flow following MH results in increased pulmonary venous return to the left heart and this response increases with GA (397). Studies indicate that a lack of vasoreactivity in response to MH may serve as a useful clinical tool in predicting lethal pulmonary hypoplasia in fetuses known to be at risk for pulmonary hypoplasia (109, 110). The development of the PA vasoreactivity to oxygen with advancing gestation has been explained by an increasing amount of smooth muscle in the small PAs (58). Our study confirms previous findings of a significant decrease in PA PI following MH in the third trimester of pregnancy (54, 109, 127).

The fetal PA AT:ET increases with advancing gestation, mainly due to a lengthening of the AT interval and a progressive decrease in PVR and an increase in pulmonary blood flow as gestation advances (53, 231). Doppler echocardiography derived PA AT correlates with invasively derived pulmonary artery pressures and PVR in children (398). The PA AT:ET is a robust measurement that is independent of HR. It is inversely correlated with

mean PA pressure in both adults and fetuses (53, 226, 399). In fact, some may argue that PA AT:ET is a better marker than PA PI and PA RI for assessing fetal PVR (226), since both the PI and RI calculations rely on the PSV and EDV measurements. The PA PSV measurement can often be inaccurate due to the varying angle of insonation between the ultrasound probe and pulmonary blood flow and the PA EDV blood flow can be minimal at the time of closure of the pulmonary arterial valve (231, 400, 401). The mean AT intervals and AT:ET in our study are consistent with prior studies at similar gestational ages (53, 226, 231). Additionally, we have demonstrated an increase in AT and in AT:ET following MH, indicating a fall in PVR.

In our study, group A comprised of fetuses at risk of pulmonary hypoplasia including those with mid-trimester PPROM and persistent oligohydramnios. We did not recruit any fetuses with a prenatal diagnosis of skeletal dysplasia or CDH as no case meeting eligibility criteria was identified over the course of the study period. The fetal pulmonary circulation is established by five weeks gestation (402). However, the critical phase in fetal lung development is between 16 and 28 weeks' gestation (403). If a PPROM occurs prior to 26 weeks gestation, fetal lung development can be impaired and this may result in pulmonary hypoplasia (404). Animal studies have emphasised the importance of mechanical factors in fetal lung development (405-409), notably the transpulmonary pressure from the permanent secretion of lung fluid and the cyclic stretch from fetal breathing movements (407). The fetal lungs remain expanded in utero due to adequate transpulmonary pressure

(410). Intrathoracic or extrathoracic compression results in varying degrees of pulmonary hypoplasia and depend on the time of onset (411) intensity, and duration (408, 412, 413). In oligohydramnios or anhydramnios, irrespective of the underlying aetiology, there is a significant degree of external pressure exerted on the fetal thorax (414, 415). Fetuses with pulmonary hypoplasia have poor pulmonary angiogenesis when compared to fetuses without pulmonary hypoplasia. The poor angiogenesis may contribute to the increased PVR and reduced pulmonary arterial compliance seen in neonates with pulmonary hypoplasia (400). The haemodynamic alterations that occur in the pulmonary vascular bed may influence pulmonary blood flow and alter the PA flow velocity waveforms on Doppler imaging.

In our study, fetuses with persistent oligohydramnios were recruited in addition to one case of mid trimester PPROM. Persistent oligohydramnios in our study was defined as a duration of ≥ seven days since the diagnosis of PPROM and oligohydramnios (DVP <2cm) in keeping with previous definitions of oligohydramnios (416, 417) on ultrasound at the time of recruitment. Most fetuses in this group (73%, n=8/11) responded to MH including the one case of mid trimester PPROM. This group was not a predictor for poorer neonatal LVCO and EF when compared with any other group. This may be due to the fact that the majority of cases (91%, n=10/11) recruited, had a PPROM confirmed in the third trimester and therefore would have a better prognosis than PPROM occurring at mid gestation. The

adverse effect of prolonged rupture of the membranes (≥ seven days) on neonatal outcomes is greatest when the PPROM has occurred prior to 26 weeks' gestation and when the duration of rupture is more than five weeks (418). The median length of time from PPROM to delivery in our study was 20 days [11-31], which may also have led to a more favourable outcome in this group.

Group B was selected as a group for recruitment because there are numerous publications that have reported higher rates of PPHN in neonates delivered by CS compared to those born by vaginal delivery (VD) (42, 419-421). Following an elective CS, both animal and human studies have demonstrated that the transductal blood flow changes from predominantly right-to-left to predominantly left-to-right within 10 minutes of birth. Therefore changes in the systemic and pulmonary vascular resistances are immediate (422, 423). It has been reported that a CS is associated with an approximately fivefold higher risk for PPHN when compared to VD (311). In addition, higher rates of RDS and concomitant increases in ET-1 levels have been reported in babies born by CS, which might indirectly lead to a higher risk of developing PPHN (424). In those babies delivered by CS that develop RDS, the requirement for mechanical ventilation is significantly higher (425). Delivery by CS also results in limited endogenous pulmonary vasodilator synthesis and lower levels of protective anti-oxidants in neonates (419). Many term infants delivered by elective CS each year require admission to

the NICU (426) with the diagnosis of TTN (427-431), RDS (311, 431-433) and severe PPHN or hypoxic respiratory failure (432, 434-436).

In our study 12 participants were recruited to group B with a median GA at delivery of 37.0 [36.8–37.2] weeks. The majority (92%, n=11/12) of participants in this group underwent a CS at less than 38 weeks GA due to an antenatal diagnosis of a placenta praevia. There is a higher risk of prematurity for babies of women with placenta praevia and concomitantly a higher risk of neonatal morbidity including RDS, low birthweight, admission to the NICU and neonatal mortality (437, 438). The increased risk of low Apgar scores and admission to the NICU remains even in term pregnancies (439). However, in our study this group was not a predictor for poorer neonatal LVCO and EF when compared with any other group.

Group C included fetuses with a prenatal diagnosis of moderate or severe perimembranous VSD or AVSD in the absence of other structural heart disease. A perimembranous VSD is the most common type of VSD, accounting for 80% of all defects and is located in the membranous septum inferior to the crista supraventricularis (440). Small VSDs with a left-to right shunt of <50% and normal pulmonary pressure are not likely to develop pulmonary hypertension or increased PVRs (441-444). During the fetal transition to neonate, uncomplicated VSDs generally do not alter the circulation significantly, with the important exception of premature infants (445). In the setting of a moderate or large VSD and with long-standing left-to-right shunting of blood, the pulmonary vascular endothelium can undergo

irreversible remodeling and dysfunction which may result in persistent pulmonary arterial hypertension (PAH) (314). However, this complication does not arise in the early newborn period and on repair of the cardiac defect, the early changes to the pulmonary vasculature are likely to be reversible. Early corrective surgery for infants (at a few months of age) will generally result in a normal PVR within one year (314, 446).

In our study, three fetuses met criteria for inclusion to group C. One neonate was lost to follow up and did not undergo a neonatal echocardiogram in our hospital. Of the two neonates that underwent a neonatal echocardiogram, both had no evidence of a VSD on neonatal imaging. The reported incidence of spontaneous VSD closure ranges from 5% to 92% and depends on the size, site, and type of defect (447, 448). Both of these VSDs were classified as moderate sized membranous VSDs at diagnosis between 20-22 weeks gestation. Perimembranous VSDs are less likely to close spontaneously than muscular VSDs (449, 450), however, in both cases in our study the VSD was no longer evident on neonatal echocardiography. Group C was not a predictor for poorer neonatal LVCO and EF when compared with any other group, however with only two patients in the group and with both showing no evidence of a VSD postnatally, it is hard to draw any meaningful conclusions from this group.

In contrast, group D was a group of 20 singleton pregnancy controls, without any documented fetal structural abnormality. Unexpectedly this group did not serve as a predictor for neonatal LVCO or EF when compared with the other

groups in the study. Of the 20 pregnant controls recruited, interestingly only 60% (n=12/20) responded to MH in utero. This interesting observation suggests that there may be an underlying predisposition to PPHN that influences risk.

4.4 Fetal Cardiac and Ductus Venosus Dopplers

4.4.1 Ductus Arteriosus

We have demonstrated no change in DA Doppler flow velocities or resistance indices in response to MH. Our data support previous reports in animal and human studies that a decrease in the pulmonary vascular impedance during MH is not caused by a constriction of the DA (54, 71, 451). During MH, the fetal DA PI and DA RI did not change. The DA PSV, EDV and TAmax did not change in response to MH in utero in our study cohort. Under normal circumstances, the fetal systemic arterial vascular resistance is lower than the pulmonary arterial vascular resistance. This results in a unidirectional shunting across the DA towards the descending aorta (54). We have demonstrated that the fetal PVR decreases in response to MH. One would anticipate that a decrease in PVR following MH would result in decreased forward flow across the DA, especially during diastole and may even lead to reversal in the direction of blood flow during diastole (138). This would result in an increased PI in the DA because the EDV and the mean velocity during the cardiac cycle decrease (54, 138). It has been

shown that fetuses with ductal constriction have higher maximal, mean and end-diastolic flow velocities and a significantly lower PI than normal fetuses (246). Therefore, a decrease in DA PI in response to MH would suggest constriction of the DA, whereas an increase in the DA PI has been found in cases with increased RVCO (246).

Rasanen at el demonstrated an increase in DA PI and DA blood flow in response to MH. In addition, they found that DA blood flow velocities and PI values returned to normal once MH was discontinued (54). Our study did not demonstrate either an increase or decrease in DA PI and is therefore not consistent with their findings. However, our findings are similar to other studies describing that changes in fetal PVR in response to MH, occur without causing ductal constriction (54, 133, 170).

Further completion of the fetal to neonatal transition is finalised by closure of the DA. This arises via multiple mechanisms with the initial functional closure occurring within a few hours after birth mainly due to smooth muscle contraction. An increase in arterial oxygen content, decreased prostaglandin levels as well as expression of ET-1 and its receptors and an increase in catecholamines all play a part in closure of the DA (452, 453). Anatomical closure of the DA can take several weeks or even months particularly in preterm infants. It is dependent upon a depletion in the arterial smooth muscle and a remodeling of the intima (454). Thus, it seems that during the short course of MH that we used, the volume of the blood flow shift from the fetal pulmonary circulation to the fetal DA was not large enough to initiate

any of the above mentioned processes, and therefore did not affect the resistance indices of the DA Doppler.

4.4.2 Aortic Isthmus

Our study has demonstrated no changes in the AoI blood flow velocities in response to MH. We did show a decrease in AoI PI however, at a p-value of 0.04, this is within the margin of error for measurements, and a reduction of 7% does not represent a meaningful clinical difference. In addition, there was no change in the AoI RI following hyperoxygenation. The fetal AoI acts as an arterial watershed between the supradiaphragmatic and infradiaphragmatic circulations (233). It is under the influence of many elements with opposing effects, including the cerebral and placental peripheral impedances and the individual performances of the right and left ventricles (455, 456). The infradiaphragmatic vascular impedances are the only influential factor during the diastolic phase of the cardiac cycle. A decrease in these impedances has many potential physiological and clinical implications (240).

The AoI Doppler waveform changes throughout gestation. In normal fetuses, in the first half of pregnancy, antegrade flow in the AoI is observed due to low placental vascular impedance. This is followed by a gradual deceleration in the systolic upstroke and a brief reversal of flow which may be related to increasing RV dominance, coupled with an increase in placental vascular

impedance as gestation advances (240, 241, 457). The timing differences between the pulmonary and aortic blood flows are the main determinants for the appearance of the end-systolic reversed flow in the AoI Doppler waveform (458). This has been explained by a delay in the onset of right ventricular ejection and in the pre-ejection period in normal fetuses (459, 460). A short peak of reversed flow in the AoI Doppler waveform in fetuses around 31 weeks gestation has been described (233, 461). The EDV or reversed flow measurements in our study did not change significantly following MH. This is in keeping with the suggestion that variations in the resistance of the fetal lungs has no influence on the appearance of the end systolic reversal peak (458).

Increased fetal cerebral blood flow can affect the right ventricular performance. We demonstrated an increase in MCA PSV in our study. Increased cerebral blood flow due to a fall in cerebral vascular resistance is always associated with an isolated rise in right ventricular preload and SV. This is due to the fact that the elevated cerebral venous return is drained by the SVC entirely into the RV (241). A major proportion of this elevated RVO goes through the DA, which is connected to the distal portion of the AoI. This will have an influence on the systolic retrograde AoI flow. We have demonstrated no change in the cerebral vascular resistance or in the AoI resistance indices in our study. Our findings are at odds with Almstrom et al who reported that in a study of 16 pregnant women, MH resulted in an increase in cerebral vascular resistance and a redistribution of blood flow

from the brain to the vascular beds supplied by the descending aorta as evidenced by a decrease in AoI PI in the third trimester (237). Our findings suggest that the fetus regulates cerebral oxygen delivery during hyperoxia, as we did not identify a significant change in the MCA PI despite an increase in the peak velocities. The reported AoI Doppler profile following MH may serve as a reference point in the future in the assessment of growth-restricted fetuses and in evaluating the utility of the AoI Doppler assessment in such cases.

4.4.3 Ductus Venosus

The changes in the DV Doppler in response to MH were characterised by an increase in the peak velocities and mean temporal velocities, in all components of the waveform. Due to the increase in systole, diastole, and atrial contraction contemporaneously, the ratios between these elements were unchanged. These findings are in keeping with those of Soregaroli et al (462). Changes in atrial pressure and volume across the systolic and diastolic phases of the cardiac cycle are reflected in variations in venous forward flow. In early systole, the AV valve rings descend rapidly. The AV valves open in early diastole and blood flows passively into the relaxed ventricles. During this time, intra-atrial pressure is low and venous forward velocity is at its highest (270). When atrial pressures are high the forward velocities decrease. The a-wave of the DV corresponds to atrial systole

when the venous forward velocity reaches the lowest point. An increase in DV blood flow velocities has been described during ventricular systole, diastole and during atrial contraction with advancing gestation (463). In addition as gestation advances the PIV and a-wave-related ratios decrease owing to a decrease in utero placental resistance and an increase in cardiac compliance and contractility (270, 274, 277, 279).

The significance of our findings is open to many interpretations, the most likely of which being a change in the size of the DV. A constriction of the DV would cause an increase in both the peak and mean temporal velocities. This hypothesis is supported by animal and human data. Animal studies have demonstrated that modifications in DV dimensions are inversely related to the UV oxygen content (464, 465). It has been demonstrated in animal data, that during fetal hypoxia, there is a significant reduction in total and umbilical venous blood flow to the liver (465). Whereas, in human studies, the size of the DV has been shown to become smaller in response to MH suggesting that the DV is responsive to alterations in oxygen tension in the fetal blood. The decrease in the DV calibre in response to MH also infers that the size of the DV is directly responsible for the shunting of umbilical venous blood away from the hepatic circulation (466).

Other plausible interpretations include a change in central venous gradient pressure or an increase in blood flow directed through the DV. However, a change in central venous gradient pressure would likely result in changes in the DV waveform and ratios (467), which we did not demonstrate in our

study. Our findings are in keeping with other human studies which observed no changes in the DV waveform ratios following MH (462, 468). Increased blood flow through the DV would seem unlikely as it has been observed that there is a decrease in blood flow through the DV at higher oxygen levels (464).

4.5 Utero-placental Dopplers

It has been hypothesised that decreased PVR may be responsible for an increase in cerebrovascular resistance (469). In our study there was no change in the MCA PI but there was evidence of improved MCA PSV parameters. This was likely due to increased pulmonary blood flow secondary to reduced fetal PVR.

Previous animal and human studies on the cerebral vascular response to MH are conflicting. Some studies have suggested an increase in cerebral vascular resistance in response to MH (282, 470), with one study demonstrating an increase in MCA PI in both the second and third trimesters (237). Other studies have demonstrated no changes in the cerebral haemodynamics following MH in healthy term pregnancies (128, 471, 472). Another study demonstrated a decrease in MCA PI in response to MH (108). Reductions in MCA PI have been described in fetuses with CHD, raising the possibility of cerebral vasodilation in response to hypoxia (473, 474).

The effects of MH on fetal haemodynamic indices are limited, owing to a relatively high fetal UV oxygen saturation during normoxia, due to the high oxygen affinity of fetal Hb (237). Maternal hyperoxygenation has been shown to increase fetal partial pressures of oxygen in the UA and UV, as well as umbilical arterial oxygen saturations (469, 475). Our findings are in keeping with other studies, where maternal hyperoxia induced no changes to UAD resistance indices (108, 110, 127, 171, 476). We have also demonstrated no change in the CPR in response to MH. The CPR is emerging as an important predictor of adverse pregnancy in IUGR fetuses but additionally in appropriately grown fetuses that are close to term (477). Appropriately grown fetuses with an abnormal CPR have higher rates of fetal distress in labour requiring emergency CS (335), lower cord pHs (478), and an increased admission rate to the NICU when compared to fetuses with a normal CPR (477).

4.6 Neonatal Response

The reactivity of the fetal PAs to hyperoxygenation and its effect on neonatal cardiac function has not been established. The results from our study represent an important change in myocardial function in the transitional period from fetal to neonatal life. This is illustrated by the increased LVCO, EF and mitral valve flow velocities in responders to MH. All fetuses in our

study were normally grown at the time of recruitment. However, there were significant postnatal differences between those that responded to MH and those that did not. The transition from the fetal to neonatal circulation can be complicated by prematurity due to the persistence of fetal shunts, high PVR as a result of RDS, increased need for mechanical ventilation, an immature myocardium and an increased incidence of metabolic acidosis (479-482). In this homogenous group of neonates, there was no difference in the GA at delivery or in the birthweight between the responders and non-responders. There was no difference in mean LV length between the two groups. Those that responded to MH, had signs of improved neonatal CO with increased LV EF and LVCO compared to non-responders. Increased mitral valve flow velocities were observed in the responder group. It is likely that increased pulmonary blood flow as a result of optimal postnatal pulmonary vasodilatation in the cohort observed to have the best response to MH prenatally, was the basis of the measured values.

For a smooth postnatal cardiovascular adaptation to occur an adequate intravascular volume is required (483). The fetal LV preload primarily depends upon the umbilical blood flow via the FO. After the umbilical cord is clamped and the lungs aerate, the neonatal LV preload primarily depends on pulmonary venous return (479). Maintaining an adequate LV preload is essential for sustaining an adequate LVO and this is the basis for the practice of delayed cord clamping (73). Physical examination is unreliable in making an accurate assessment of neonatal cardiac function and

haemodynamics in sick neonates (484). Cardiac preload can be estimated by measuring LV volumes in neonates (479). There is a doubling of LVO in normal neonates, within the first hour of life (68). In our study, the LV end-diastolic area and volume were measured using the Simpson's biplane method in keeping with international guidance (485). Delayed cord clamping is not routinely employed in our hospital for term babies being delivered by CS. It typically occurs inadvertently following vaginal deliveries, however, neonatal LVCO was not dependent on mode of delivery in our study. Neonatal LVCO volumes in our study were similar to those reported in other appropriately grown fetuses in the first day of life (483), but lower than in other studies (486-488). The slightly lower overall LVCO volumes in the presence of a normal EF indicates that the altered LVCO is more likely explained by changes of vascular resistance than by contractility.

The most immediate and adverse adaptation of the fetal to neonatal transition is the persistence of high PVR (479). This can result in continued right to left or bidirectional shunting across the DA and/or FO leading to reduced pulmonary blood flow. Reduced pulmonary blood flow generates decreased pulmonary venous return and accordingly decreased LV preload and low LVCO (489). If this cycle continues, it results in decreased organ perfusion, an increase in lactate, acidosis and hypoxia which are potent pulmonary vasoconstrictors and ultimately can lead to the development of pulmonary hypertension (8, 482). The only predictor of a reduced neonatal LVCO in our study was the fetal response to MH in utero. The LVCO was not

dependent on mode of delivery or GA at delivery and was not dependent on FHR. This interesting observation suggests that the neonatal compromise due to CS is primarily a respiratory problem and that there is a cohort of infants who may be identified by MH prenatally, who would be predicted to have an increased vulnerability to deal with neonatal illness, due to a limited ability to achieve the pulmonary vasodilatation necessary to optimise LV preload and therefore increase their CO.

In relation to neonatal EF, in our study the fetal response to MH was predictive of the measured neonatal EF and was not dependent on GA at delivery or mode of delivery. The EF was unsurprisingly dependent on the FHR because it is a measure of the proportion of diastolic volume ejected during ventricular contraction.

The neonatal EF reflects SV assessed on echocardiography. It's measurement is preferred over measurement of the fractional shortening, as it accounts for regional wall motion abnormalities, and in most instances is easy to acquire (490). Measured EF has good correlation with invasive measures of LV function longitudinally, and its clinical utility in paediatrics has been established in multiple studies (491, 492). Normative values for EF in neonates have been established (479). A normal neonatal EF is >55%, a slightly reduced EF is classified as 41–55%, a moderately reduced EF is classed as 31–40%, and a markedly reduced EF is classed as ≤30% (493, 494). In our study, the mean EF in the non-responders was 47 ± 7% which

suggests that all values were in the slightly reduced group in comparison to the responders, where the mean EF was 54 ±9%.

If there is a failure of the normal neonatal decrease in PVR, the RV plays an important role in adapting to variations in pulmonary blood flow (281). In our study, there were no differences in nPAAT, RV end systolic pressures or in PDA characteristics between the two groups. This is likely due to the fact that the PDA usually closes within 24–48 hours after birth in full-term infants (495) and the neonatal echocardiographs in our study were all performed before 24 hours of life. Therefore, this may have influenced measured RV indices. In a term infant, the functional closure of the DA occurs within 24 to 48 hours after birth while anatomical closure may take up to 10 to 14 days (496). The FO can remain patent for some years (497). Therefore, the RV cardiac parameters would likely demonstrate changes at a later point, within 48 hours after birth (498).

4.7 Repeatability

We have demonstrated low intraobserver and interobserver variability of PA PI and PA RI, with mean percent errors ranging from 4% to 6%. We have demonstrated high levels of repeatability and ICC for inter and intraobserver measures. Measurements of different fetal blood velocity waveforms can introduce a potential error due to inter and intraobserver variability. Our findings are in keeping with previous studies that have shown excellent

repeatability of PA Doppler waveform indices (346) with intraobserver variability for PA PI values of between 4%-12% and ICC of 95% (54, 223, 499). We calculated the repeatability by the mean and the SD of differences and the repeatability coefficient of the two repeated tests within patients, as has been previously described (223, 317). From a clinical perspective, the high-pass filter rate of 100Hz may have hidden minimal retrograde flow in the PA in some cases, but we did not wish to use the lowest high-pass filter rate of 50Hz as this may have induced considerable interference from the PA vessel wall. Sampling at the DPA as compared to the proximal PA may produce a lower PSV and overall time of the systolic component (500). However, as PVR is high in utero, this would be recorded as early diastolic retrograde flow (501) and would not have any overall effect on measured values providing there was consistency in the Doppler gate placement.

4.8 Feasibility, Acceptability, Safety

4.8.1 Feasibility

This feasibility study has found that pregnant women in the third trimester were open to undergoing MH with high levels of participation. Additionally, they were willing to undergo the study procedure again in the future. Of all potential participants that were approached, only four pregnant subjects (7%, n=4/55) declined participation. Our findings are in keeping with others that have demonstrated a high willingness in pregnant women to participate in

trials (502, 503). Our study provided a potential benefit to participants, in that their infant would undergo a neonatal echocardiogram prior to discharge from hospital. This could have potentially identified a cardiac issue that had not previously been diagnosed on prenatal ultrasound or on routine physical examination. A potential benefit to the health of the fetus has been previously described as the most important determinant for willingness of pregnant women to partake in trials (502, 504), and this may be the reason that we had a very high rate of participation. All non-pregnant subjects that were approached participated in the study. This illustrates high levels of acceptability of the study procedure in non-pregnant and pregnant subjects.

The lower than anticipated number of participants in group A is as a consequence of the natural history of PPROM, in that 83%, (n=5/6) of subjects with mid-trimester PPROM who had initially consented to partake in the study, delivered prior to 31 weeks' GA. I performed a retrospective analysis of the Rotunda Hospital annual data for the years January 1st to December 31st 2017 and 2018 and found that there were 20 and 21 mid-trimester cases of PPROM respectively during those years.

Additionally, I performed a retrospective review of all moderate and severe perimembranous VSD and AVSD cases, in the absence of any other structural abnormality and in normally grown fetuses, attending the Rotunda Hospital over the study period. Nine cases meeting this group's eligibility criteria were identified, including the five cases that were screened in the

study. The low throughput of potential participants in both groups raises concerns in relation to the scheduling considerations for a larger study.

We aimed to ascertain the proportion of women in whom it was possible to obtain a PA Doppler velocimetry measurement and we have demonstrated high levels of PA Doppler acquisition. This is in keeping with the published literature (101, 223, 225, 231, 400, 401). Rates of technical ease in accusation of PA Doppler waveforms were either very easy or easy in the majority of cases.

Rates of acquisition for other cardiac Dopplers including the DA and AoI were high in our study. High rates of DA Doppler acquisition have been previously reported (246, 338). There is limited use of the AoI Doppler in routine clinical practice in the assessment of fetal growth restriction, mainly due to the technical difficulty in obtaining the waveform. However it has been shown that adequate imaging of the fetal AoI can be achieved easily by trained sonographers (455) and our findings are in keeping with this study.

Rates of adherence to the study protocol were high with all participants completing the hyperoxygenation phase and subsequent ultrasound examinations and nearly three-quarters of all neonates undergoing an echocardiogram. Almost all patient outcomes were recorded and only two neonatal follow-ups were unable to be completed. Therefore, our findings are in keeping with others in concluding that administering MH in pregnancy is feasible (170, 397).

In relation to the economic considerations of the study, the overall fixed costs are low and once a NICOM® machine is purchased, it can be used on all study participants. Our economic analysis did not take into account the cost of training staff in performing fetal or neonatal echocardiography or staff time implications as this is extremely variable and difficult to determine.

4.8.2 Acceptability

There have been few studies assessing the acceptability of non-routine medications in pregnancy (344). We have demonstrated high patient satisfaction rates in relation to the non-routine administration of oxygen in pregnancy and the timing of the overall study procedure. There were high rates of acceptability for a repeat procedure. All participants in our study found that oxygen administered via a non-rebreather mask was comfortable. A previous study had reported that oxygen administered by a facemask was less acceptable than by nasal cannulae and the authors concluded that the facemask impedes maternal ability to communicate and that nasal cannulae are more acceptable if oxygen therapy is indicated (387). However, this was in the setting of an elective CS and not for the purposes of assessing a fetal response to hyperoxia.

4.8.3 Safety

An important aspect to this thesis was to highlight the measures taken to ensure safety and wellbeing of participants involved in the study. Reporting of adverse events was fundamental to detecting participant safety issues. We employed the use of the NICOM® technology to continuously assess maternal and non-maternal haemodynamic changes in response to hyperoxygenation administration. We have reported no maternal or non-maternal side effects from acute hyperoxygenation which is consistent with the published literature (127, 170, 397). There has been previously one report of epistaxis on the first day of chronic MH therapy, which resolved with the addition of a humidifier to the condensing unit, in one study (170).

The routine monitoring methods employed on labour wards in the assessment of the pregnant woman, include BP monitoring with a manual or automated BP cuff and measurement of the HR. Based on these proxy measures, we gain very little insight into the true circulatory response to any treatment or measure aimed at increasing the CO or oxygen delivery to the fetus. The NICOM® system offers the capability to accurately reflect such circulatory changes beyond the analysis of vital signs alone. We have demonstrated an increase in SVR which coincided with an acute reduction in CI in both pregnant and non-pregnant participants. These haemodynamic changes did not recover to baseline levels following the cessation of hyperoxygenation. This raises a safety issue in relation to many pregnancy disease states such as gestational hypertension and preeclampsia as well

as some forms of maternal cardiac disease where an increase in SVR and a reduction in CI may not be desirable, in particular during times of exertion such as during labour where coincidentally the administration of oxygen is often initiated.

4.9 Strengths

Feasibility studies represent a fundamental phase of the research process. It is important to bear in mind that a feasibility study is not a hypothesis testing study. A strength of our study relies on the fact that it was a prospective study in a single tertiary referral site, which offers a more realistic examination of recruitment and implementation of the MH test in this setting.

To our knowledge, this is the first study using direct maternal haemodynamic measurements to evaluate the effects of hyperoxygenation in pregnancy. The NICOM® system that was employed is entirely operator independent and is therefore not subject to any interobserver variation or bias. Additionally, to the best of our knowledge, this is the first prospective study to investigate the fetal response to MH in terms of correlating multiple fetal cardiac Dopplers with neonatal echocardiographic indices, in appropriately grown fetuses in the third trimester of pregnancy.

A main strength of the study is that all the ultrasound and Doppler measurements were performed by a single operator (A.M).

There is no doubt that this feasibility study has provided pivotal data in the ability to recruit the desired number of participates required for a potential definitive study and in the timing needed to achieve that. The data analysis from this feasibility work has proven highly informative in relation to the ability to obtain fetal cardiac Doppler information in the third trimester. We would anticipate a high rate of participation at this hospital with demonstrable high compliance levels with the study protocol and the study procedure if a larger trial was to proceed.

4.10 Limitations

We acknowledge the small sample size in our study. Although our findings suggest a correlation between some fetal cardiac Dopplers and neonatal cardiac indices, the study was not powered as a predictive tool and thus may not be reflective of a cause and effect relationship. This warrants further exploration in a larger cohort to establish the ability of the fetal response to MH to predict postnatal adaptation of the pulmonary vascular circulation, during the transition to neonatal life. Measurements of circulating antioxidants or markers of oxidative damage were also not obtained.

We acknowledge the limitations in the wider application of the assessment of fetal and neonatal cardiac function outside of tertiary centres and research studies, as advanced sonographic skills are required.

The administration of antenatal corticosteroids are known to protect against the development of RDS in infants delivered before 34 weeks' gestation (505). Whether they alter PA waveform measurements has not been systematically examined. We recognise that 54% of the study participants had received antenatal steroids prior to the PA ultrasonographic measurements and it is unknown whether this would influence our results.

4.11 Research objectives achieved

Five research objectives were identified at the start of this research project as follows:

1. To assess feasibility of recruitment, safety, and acceptability of the hyperoxygenation test in pregnant women.

2. To evaluate the fetal response to maternal hyperoxygenation in singleton pregnancies in the third trimester.

3. To evaluate if pulmonary artery reactivity to maternal hyperoxygenation identifies fetuses that will develop pulmonary hypertension in the early newborn period.

4. To correlate neonatal echocardiographic findings with fetal echocardiographic changes in response to maternal hyperoxygenation.

5. To assess serial changes in maternal cardiac output, stroke volume and systemic vascular resistance before, during and after maternal hyperoxygenation.

These objectives have been achieved over the course of this thesis

To assess feasibility of recruitment, safety, and acceptability of the hyperoxygenation test in pregnant women.

We have illustrated high levels of acceptability of the study procedure and of the administration of MH in pregnancy. We have reported that recruitment is feasible and identified some areas which may affect the feasibility of recruitment in any future study. Our findings question the safety of hyperoxygenation use in a non-hypoxemic pregnant patient. Hyperoxygenation may counteract any intended increase in oxygen delivery and may cause harm. We recommend that further investigation into the benefits and risks of MH in an obstetric population is performed.

To evaluate the fetal response to maternal hyperoxygenation in singleton pregnancies in the third trimester.

Our findings indicate a reduction in fetal PVR with a resultant increase in fetal pulmonary blood flow, increased left atrial return and increased LVCO.The decrease in PVR was characterised by a decrease in the PA PI and PA RI and an increase in the PA AT:ET following MH. These changes did not occur at the expense of ductal constriction nor did they result in any significant changes in the AoI Doppler waveform. Maternal hyperoxygenation was not associated with any changes in the utero-placental circulation

indices. Although, MH led to an increase in the peak velocities and mean temporal velocities of the DV Doppler waveform, it did not alter the DV velocity ratios. No changes were identified in fetal HR in response to MH.

To evaluate if pulmonary artery reactivity to MH identifies fetuses that will develop pulmonary hypertension in the early newborn period.

Despite the fact that no neonate in our study was diagnosed with PPHN, there were significant neonatal echocardiographic differences in those that responded to MH when compared to those that did not. We have demonstrated that the degree of the fetal response to MH in utero may resemble the rate of transition during the early neonatal period. Our study suggests that the prenatal hyperoxygenation test may offer the potential to predict an optimal adaptation to postnatal life with rapid postnatal reduction in PVR increasing measured cardiac output. The clinical implications of these findings require further investigation in relation to pathologic conditions, such as lung hypoplasia and congenital cardiac disease in greater numbers, before ascertaining whether it can accurately identify neonates that will develop PPHN.

To correlate neonatal echocardiographic findings with fetal echocardiographic changes in response to maternal hyperoxygenation.

We have shown that an appropriate response to MH in utero was reflective of an optimal adaptation to postnatal life. This was demonstrated by an increased capacity in fetuses that responded to MH prenatally, to increase their pulmonary blood flow, decrease PVR, increase pulmonary venous return and increase left ventricular preload. This resulted in an increased left ventricular EF and LVCO during the early neonatal period, in fetuses that were classified as responders to MH when compared to non-responders. The neonatal echocardiographic changes in responders were due to an increase in mitral flow velocity (likely reflecting increased pulmonary blood flow) and not related to an increase in left ventricular size.

To assess serial changes in maternal cardiac output, stroke volume and systemic vascular resistance before, during and after maternal hyperoxygenation.

We have demonstrated that hyperoxygenation use in pregnancy resulted in a fall in CO and CI which coincided with a rise in SVR. These findings were more pronounced in the pregnant group versus the changes observed in the non-pregnant group exposed to the same duration and dose of oxygen.

4.12 Conclusion

This study supports the contention that the prenatal hyperoxygenation test may offer the potential to predict an optimal adaptation to postnatal life. As in any newborn, the capacity to optimally reduce PVR postnatally is likely to have an important role in increasing LV preload and ultimately LV cardiac output. We have shown that it is possible to obtain PA Doppler velocity waveforms and that in the third trimester of pregnancy the fetal pulmonary arterial circulation is dynamic and can reflect acute changes in fetal oxygenation. The clinical implications of these findings require further investigation particularly in the context of pathologic conditions such as lung hypoplasia and congenital cardiac disease.

In light of the observed pregnancy specific reaction to hyperoxygenation which is not reflected in BP and HR measurements, maternal administration of high oxygen concentrations should be undertaken judiciously and with appropriate monitoring. The findings of this thesis, indicating that hyperoxygenation in the non-hypoxemic pregnant woman may have unintended and potentially detrimental haemodynamic effects, warrant further exploration in a larger cohort. Importantly, these changes need further evaluation in disease states such as pre-eclampsia, as the changes in that population may be more profound and with potentially deleterious consequences. Oxygen supplementation in the setting of hypoxia is clearly justified. However, we must caution its use in normoxic pregnant women until randomised controlled trial evidence is available to support its use.

These studies are imperative as hyperoxia confers a theoretic potential to cause harm. The precise role of hyperoxygenation therapy in an obstetric population should be carefully evaluated.

4.13 Future considerations

The findings of this work extend our understanding of fetal physiology in the third trimester. This work provides insights that can be used to further evaluate fetal and neonatal cardiac function. It is clear that further research is warranted in this area. We believe that a method that evaluates fetal pulmonary vascular function may prove to be a more reliable predictor of postnatal cardio-pulmonary dysfunction compared to the prenatal evaluation of anatomic structures alone.

Newer methods of non-invasive assessment of fetal lung maturity have been recently assessed, including quantitative ultrasound texture analysis of the fetal lung (quantusFLM) (506) and this appears promising. Ultimately, the ability to predict neonatal respiratory morbidity with acceptable accuracy would enable clinicians and parents to make balanced and informed decisions in relation to the management of the pregnancy, including the most appropriate place for delivery. In particular, for high risk cases including fetuses with mid-trimester PPROM, a predictive assessment of neonatal lung function that could be performed in the fetal period would allow ample time for delivery planning and would help guide the pharmacological and neonatal

ICU strategies that could optimise postnatal survival. The use of MH for this purpose warrants further exploration in a larger cohort.

NICOM® has been validated for use in the pregnant population and it presents an opportunity for further non-invasive interrogation of maternal haemodynamics. This novel technology may allow us to further evaluate the haemodynamic response to oxygen administration in pregnancy in a wide variety of clinical settings and in different pregnancy disease states.

We aspire to continue our investigation into the maternal and fetal effects of oxygen use in pregnancy. We believe that further studies using MH should be considered carefully and have a well-structured methodology and power. Only then can we truly ascertain the magnitude of the effect of hyperoxygenation use in pregnancy on both mother and fetus. Finally, we have a responsibility to accelerate the implementation of proven innovative therapies and diagnostic tools to realise of the benefits of research for individual patients and their families.

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6 Appendices

An tOspidéal Náisiúnta Máithreachais
The National Maternity Hospital
Founded in 1894

Sráid Holles, Baile Átha Cliath 2 • Holles Street, Dublin 2
Telephone: (01) 6373100. Fax: 6766623. Web: www.nmh.ie

Máistir/ Master: Dr. Rhona Mahony

PRIVATE AND CONFIDENTIAL

Professor Fionnuala Breathnach,
Associate Professor Obstetrics and Gynaecology,
Royal College of Surgeons in Ireland,
Rotunda Hospital,
Parnel Square,
Dublin 1.

13th September 2016

Our ref: **EC 28.2016**

Re: **Can sonographic assessment of pulmonary vascular reactivity following the maternal hyperoxygenation test predict neonatal outcome in foetuses at risk of pulmonary hypertension?**

Dear Professor Breathnach,

The above study was approved by the ethics committee on the 05th September 2016.

We wish you success with the study.

Kind regards,
Yours sincerely,

Dr. John Murphy
Chairman,
Ethics Research Committee

Cc. Dr. Ann McHugh, Specialist Registrar in Obstetrics and Gynaecology, Royal College of Surgeons in Ireland, Master's House, Rotunda Hospital, Parrell Square, Dublin 1.

Participation Information Leaflet

Thank you for taking the time to read this Information Leaflet

Title of the Research Project

Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension

Who are the Researchers?

Lead Researcher: Dr Ann McHugh, SpR Obstetrics and Gynecology, Rotunda Hospital **Telephone No: 01- 4022535.**

Principal Investigators: Professor Fionnuala Breathnach, Obstetrics and Gynecology, Rotunda Hospital – RCSI unit. Dr. Orla Franklin, Neonatal cardiology, Our Lady's Hospital for Sick Children, Dublin/ The Rotunda Hospital, Dublin. Dr Afif El-Khuffash, Clinical Director, Neonatology, Rotunda Hospital

What is this Research Project about?

We are investigating a medical condition called **P**ersistent **P**ulmonary **H**ypertension of the **N**ewborn (PPHN). This is a medical problem that can develop after the baby is born and while in the newborn period. The condition can lead to serious respiratory (breathing) and cardiovascular (heart) problems in the newborn.

Recent studies have shown that there is a test that can be performed during pregnancy to predict which newborns are likely to develop the condition and which newborns will develop a severe form of PPHN. By examining how the fetal heart (the 4 pumping chambers of the heart and the vessels coming out of the heart that supply the body) reacts in the womb to oxygen given to the mother, we may be

able to predict more accurately which babies will develop PPHN and which of those will have a severe form of the condition.

PPHN occurs in 0.5-7 per 1000 live births when the normal circulatory (heart) transition in the early newborn period fails to occur. If we knew before delivery which babies would suffer with this condition, it would allow us put in place the most effective management plan for both mother and baby.

Why am I being asked to participate in this research project?

You are being asked to participate in this study because your pregnancy has been complicated by one of the following conditions:
Mid trimester premature rupture of membranes (PPROM)
Reduced amniotic fluid levels
Your baby has been diagnosed with Congenital diaphragmatic hernia (CDH), Congenital cystic adenomatoid malformation (CCAM) or another space occupying lesions of the thorax (cardiomegaly, pleural effusion, and skeletal dysplasia).

These conditions can increase the risk of your baby developing PPHN. By participating in the study we will gain more detailed information on how babies with these particular conditions adapt to the oxygen test in the womb. We hope that this will enable us to predict more accurately the occurrence and severity of PPHN.

What will be my role in this Research Project?

The current standard of care at the Rotunda Hospital is that all pregnancies undergo a 20 week anatomy scan which includes an assessment of the fetal heart. Participating in this research project will involve one additional ultrasound scan of the baby's wellbeing and a more detailed examination of the fetal heart. The baby's heart will also be scanned after it is born.

However, before you decide whether or not to take part, it is important that you fully understand what the research is about and what you will be asked to do. It is important that you read the information below in order to make an informed decision and if you have any questions about any aspects of the study that are not clear to you, do not hesitate to ask me. Please make sure that you are satisfied

before you decide to take part or not. It is important for you to know that deciding not to take part in this study will not affect your normal treatment in the Rotunda Hospital in any way

If you agree to participate you will be invited for an additional ultrasound scan of your baby's heart during your pregnancy (after 31 weeks of gestation). The scan will be undertaken in the fetal assessment unit (FAU) located on the second floor of the hospital. A qualified healthcare professional with expertise in maternal-fetal medicine and fetal and neonatal cardiology will perform your scan (the lead researcher and the principal investigators listed on this form).

Immediately following this, we will administer an oxygen test. This involves fitting you with a face mask which will supply oxygen for a total duration of ten minutes. Oxygen is present in the air we breathe and is safe to use in pregnancy. The reason we administer the oxygen through a face mask is to allow a higher flow rate (amount) to be given to you over a short period of time. The face mask is a plastic mask which will be fitted to cover over your nose and mouth. During this time you be lying down on the normal couch in the ultrasound department. Four stickers will be placed on your back to allow us to detect any changes in your own blood pressure or heart rate during the oxygen test. After ten minutes, a repeat ultrasound of the fetal heart will be performed. We will examine how the fetal heart has reacted to the oxygen test and this will hopefully allow us to understand better how that particular baby will adapt to newborn life and whether or not they will develop PPHN. The entire duration of your visit will be approximately 30 minutes.

As mentioned earlier a similar scan of your baby's heart will be performed on the baby after delivery. This will assess for the presence and severity of PPHN. All pregnancies will be followed up for outcomes at delivery.

Are there any Potential Harms/Risks?

Fetal and neonatal ultrasound is not associated with any identifiable maternal or fetal risks and there are no known risks to you or your baby when participating in this study.

Oxygen is safe to use in pregnancy, and high flow oxygen given for ten minutes duration is not known to be associated with any risks to you or your baby. Your own cardiovascular response will be monitored throughout the test. If for any reason you would like us to stop the test at any time we will do so.

In the rare event that the Fetal Medicine doctor performing your study examination identifies a problem during the study scan that could require a change in your pregnancy management (for example, evidence of deteriorating function of the placenta), she will alert your doctor/ medical team immediately, to coordinate on-going management plans for your pregnancy. Participation in this study will not affect the management of your pregnancy or the care of your baby, and responsibility for all clinical decisions in relation to your care and that of your baby will remain with your treating doctor and clinical team.

Are there any Potential Benefits?

Participation in this study is not likely to be associated with any specific benefit to you or your baby. However, you will receive an additional ultrasound scan which will assess fetal wellbeing and provide a more detailed cardiac assessment. Your baby will undergo a heart ultrasound when born which could potentially identify a cardiac problem at a very early stage.

What measures will be taken to ensure confidentiality?

We will respect completely your right to confidentiality. All data will be processed and analysed without being labelled with your name or medical history, and will be anonymized at the earliest opportunity. Regarding medical details and paper records obtained, these will be kept in a locked room and all electronic scan data will be stored on a single, password protected encrypted computer drive during the analysis of the data in the Rotunda Hospital. Data will be stored for a maximum of 5 years and will be destroyed confidentially after the study has been completed and published.

Other Considerations

We will require access to your medical records and those of your baby for the purpose of the research to collect data in relation to your pregnancy and its outcome and you are asked to consent to this information being collected and used for the purpose of this project. Your medical records will not leave the Rotunda Hospital and will be made available for you and your baby at each of your hospital visits.

Before you make a decision to participate or not, please read carefully the information provided and take the time to ask questions. If you wish to discuss any aspect of the study with any of the study researchers or with family and friends, or your GP please take the time to do so.

You may change your mind at any time and decide to withdraw before the start of the study or even after the study has commenced. You do not have to justify your decision to any person involved in the study. Your decision to participate or not in the research will have no effect on your care or the care of your baby in the hospital. If you have any further questions about the study, you may contact the lead researcher at the number below:
Dr Ann McHugh 01- 4022535.

At the completion of the project, will I be informed of the research outcome?

If you would like to know about the results of the research please let Dr Ann McHugh know. The results will be presented at scientific meetings and will be published in a medical journal but, again, we stress that no information will be released which would in any way identify you.

My rights as a Participant

1. I am informed that no information regarding my medical history will be divulged and the results of any tests involving myself will not be published so as to reveal my identity.
2. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to myself.
3. I understand that this research project has been approved by the relevant hospital Ethics Committee
4. I have been offered a copy of this document for my records

Participation Information Leaflet

Thank you for taking the time to read this Information Leaflet

Title of the Research Project

Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension

Who are the Researchers?

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Principal Investigators: Professor Fionnuala Breathnach, Obstetrics and Gynecology, Rotunda Hospital – RCSI unit. Dr. Orla Franklin, Neonatal cardiology, Our Lady's Hospital for Sick Children, Dublin/ The Rotunda Hospital, Dublin. Dr Afif El-Khuffash, Clinical Director, Neonatology, Rotunda Hospital

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Recent studies have shown that there is a test that can be performed during pregnancy to predict which newborns are likely to develop the condition and which newborns will develop a severe form of PPHN. By examining how the fetal heart (the 4 pumping chambers of the heart and the vessels coming out of the heart that supply the body) reacts in the womb to oxygen given to the mother, we may be

able to predict more accurately which babies will develop PPHN and which of those will have a severe form of the condition.

PPHN occurs in 0.5-7 per 1000 live births when the normal circulatory (heart) transition in the early newborn period fails to occur. If we knew before delivery which babies would suffer with this condition, it would allow us put in place the most effective management plan for both mother and baby.

Why am I being asked to participate in this research project?

You are being asked to participate in this study because you are undergoing a scheduled caesarean section before 38 weeks gestation.

Elective caesarean section before 38 weeks gestation in an otherwise healthy baby can increase the risk of PPHN. By participating in the study we will gain valuable information in relation to the baby's circulatory (heart) adaptation close to term.

What will be my role in this Research Project?

The current standard of care at the Rotunda Hospital is that all pregnancies undergo a 20 week anatomy scan which includes an assessment of the fetal heart. Participating in this research project will involve one additional ultrasound scan of the baby's wellbeing and a more detailed examination of the fetal heart. The baby's heart will also be scanned after it is born.

However, before you decide whether or not to take part, it is important that you fully understand what the research is about and what you will be asked to do. It is important that you read the information below in order to make an informed decision and if you have any questions about any aspects of the study that are not clear to you, do not hesitate to ask me. Please make sure that you are satisfied before you decide to take part or not. It is important for you to know that deciding not to take part in this study will not affect your normal treatment in the Rotunda Hospital in any way

If you agree to participate you will be invited for an additional ultrasound scan of your baby's heart during your pregnancy (after 31 weeks of gestation). The scan will be undertaken in the fetal assessment unit (FAU) located on the second floor of the hospital. A qualified healthcare professional

with expertise in maternal-fetal medicine and fetal and neonatal cardiology will perform your scan (the lead researcher and the principal investigators listed on this form).

Immediately following this, we will administer an oxygen test. This involves fitting you with a face mask which will supply oxygen for a total duration of ten minutes. Oxygen is present in the air we breathe and is safe to use in pregnancy. The reason we administer the oxygen through a face mask is to allow a higher flow rate (amount) to be given to you over a short period of time. The face mask is a plastic mask which will be fitted to cover over your nose and mouth. During this time you be lying down on the normal couch in the ultrasound department. Four stickers will be placed on your back to allow us to detect any changes in your own blood pressure or heart rate during the oxygen test. After ten minutes, a repeat ultrasound of the fetal heart will be performed. We will examine how the fetal heart has reacted to the oxygen test and this will hopefully allow us to understand better how that particular baby will adapt to newborn life and whether or not they will develop PPHN. The entire duration of your visit will be approximately 30 minutes.

As mentioned earlier a similar scan of your baby's heart will be performed on the baby after delivery. This will assess for the presence and severity of PPHN. All pregnancies will be followed up for outcomes at delivery.

Are there any Potential Harms/Risks?

Fetal and neonatal ultrasound is not associated with any identifiable maternal or fetal risks and there are no known risks to you or your baby when participating in this study.

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Why am I being asked to participate in this research project?

You are being asked to participate in this study because your baby has been diagnosed with either a moderate/severe perimembranous ventricular septal defect (VSD) or an atrioventricular septal defect (AVSD), in the absence of any other structural heart disease.

By participating in the study we will gain valuable information in relation to how these babies adapt in the womb to the oxygen test. We hope that this will allow us to predict which babies will develop PPHN and of those who do, which will develop a severe form of the condition.

What will be my role in this Research Project?

The current standard of care at the Rotunda Hospital is that all pregnancies undergo a 20 week anatomy scan which includes an assessment of the fetal heart. Participating in this research project will involve one additional ultrasound scan of the baby's wellbeing and a more detailed examination of the fetal heart. The baby's heart will also be scanned after it is born.

However, before you decide whether or not to take part, it is important that you fully understand what the research is about and what you will be asked to do. It is important that you read the information below in order to make an informed decision and if you have any questions about any aspects of the study that are not clear to you, do not hesitate to ask me. Please make sure that you are satisfied before you decide to take part or not. It is important for you to know that deciding not to take part in this study will not affect your normal treatment in the Rotunda Hospital in any way

If you agree to participate you will be invited for an additional ultrasound scan of your baby's heart during your pregnancy (after 31 weeks of gestation). The scan will be undertaken in the fetal

assessment unit (FAU) located on the second floor of the hospital. A qualified healthcare professional with expertise in maternal-fetal medicine and fetal and neonatal cardiology will perform your scan (the lead researcher and the principal investigators listed on this form).

Immediately following this, we will administer an oxygen test. This involves fitting you with a face mask which will supply oxygen for a total duration of ten minutes. Oxygen is present in the air we breathe and is safe to use in pregnancy. The reason we administer the oxygen through a face mask is to allow a higher flow rate (amount) to be given to you over a short period of time. The face mask is a plastic mask which will be fitted to cover over your nose and mouth. During this time you be lying down on the normal couch in the ultrasound department. Four stickers will be placed on your back to allow us to detect any changes in your own blood pressure or heart rate during the oxygen test. After ten minutes, a repeat ultrasound of the fetal heart will be performed. We will examine how the fetal heart has reacted to the oxygen test and this will hopefully allow us to understand better how that particular baby will adapt to newborn life and whether or not they will develop PPHN. The entire duration of your visit will be approximately 30 minutes.

As mentioned earlier a similar scan of your baby's heart will be performed on the baby after delivery. This will assess for the presence and severity of PPHN. All pregnancies will be followed up for outcomes at delivery.

Are there any Potential Harms/Risks?

Fetal and neonatal ultrasound is not associated with any identifiable maternal or fetal risks and there are no known risks to you or your baby when participating in this study.

Oxygen is safe to use in pregnancy, and high flow oxygen given for ten minutes duration is not known to be associated with any risks to you or your baby. Your own cardiovascular response will be monitored throughout the test. If for any reason you would like us to stop the test at any time we will do so.

In the rare event that the Fetal Medicine doctor performing your study examination identifies a problem during the study scan that could require a change in your pregnancy management (for example, evidence of deteriorating function of the placenta), she will alert your doctor/ medical team immediately, to coordinate on-going management plans for your pregnancy. Participation in this study will not affect the management of your pregnancy or the care of your baby, and

responsibility for all clinical decisions in relation to your care and that of your baby will remain with your treating doctor and clinical team.

Are there any Potential Benefits?

Participation in this study is not likely to be associated with any specific benefit to you or your baby. However, you will receive an additional ultrasound scan which will assess fetal wellbeing and provide a more detailed cardiac assessment. Your baby will undergo a heart ultrasound when born which could potentially identify a cardiac problem at a very early stage.

What measures will be taken to ensure confidentiality?

We will respect completely your right to confidentiality. All data will be processed and analysed without being labelled with your name or medical history, and will be anonymized at the earliest opportunity. Regarding medical details and paper records obtained, these will be kept in a locked room and all electronic scan data will be stored on a single, password protected encrypted computer drive during the analysis of the data in the Rotunda Hospital. Data will be stored for a maximum of 5 years and will be destroyed confidentially after the study has been completed and published.

Other Considerations

We will require access to your medical records and those of your baby for the purpose of the research to collect data in relation to your pregnancy and its outcome and you are asked to consent to this information being collected and used for the purpose of this project. Your medical records will not leave the Rotunda Hospital and will be made available for you and your baby at each of your hospital visits.

Before you make a decision to participate or not, please read carefully the information provided and take the time to ask questions. If you wish to discuss any aspect of the study with any of the study researchers or with family and friends, or your GP please take the time to do so.

You may change your mind at any time and decide to withdraw before the start of the study or even after the study has commenced. You do not have to justify your decision to any person involved in

the study. Your decision to participate or not in the research will have no effect on your care or the care of your baby in the hospital. If you have any further questions about the study, you may contact the lead researcher at the number below:
Dr Ann McHugh 01- 4022535.

At the completion of the project, will I be informed of the research outcome?

If you would like to know about the results of the research please let Dr Ann McHugh know. The results will be presented at scientific meetings and will be published in a medical journal but, again, we stress that no information will be released which would in any way identify you.

My rights as a Participant

1. I am informed that no information regarding my medical history will be divulged and the results of any tests involving myself will not be published so as to reveal my identity.
2. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to myself.
3. I understand that this research project has been approved by the relevant hospital Ethics Committee
4. I have been offered a copy of this document for my records

Participation Information Leaflet

Thank you for taking the time to read this Information Leaflet

Title of the Research Project

Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension

Who are the Researchers?

Lead Researcher: Dr Ann McHugh, SpR Obstetrics and Gynecology, Rotunda Hospital **Telephone No: 01- 4022535.**

Principal Investigators: Professor Fionnuala Breathnach, Obstetrics and Gynecology, Rotunda Hospital – RCSI unit. Dr. Orla Franklin, Neonatal cardiology, Our Lady's Hospital for Sick Children, Dublin/ The Rotunda Hospital, Dublin. Dr Afif El-Khuffash, Clinical Director, Neonatology, Rotunda Hospital

What is this Research Project about?

We are investigating a medical condition called **P**ersistent **P**ulmonary **H**ypertension of the **N**ewborn (PPHN). This is a medical problem that can develop after the baby is born and while in the newborn period. The condition can lead to serious respiratory (breathing) and cardiovascular (heart) problems in the newborn.

Recent studies have shown that there is a test that can be performed during pregnancy to predict which newborns are likely to develop the condition and which newborns will develop a severe form of PPHN. By examining how the fetal heart (the 4 pumping chambers of the heart and the vessels coming out of the heart that supply the body) reacts in the womb to oxygen given to the mother, we

may be able to predict more accurately which babies will develop PPHN and which of those will have a severe form of the condition.

PPHN occurs in 0.5-7 per 1000 live births when the normal circulatory (heart) transition in the early newborn period fails to occur. If we knew before delivery which babies would suffer with this condition, it would allow us put in place the most effective management plan for both mother and baby.

Why am I being asked to participate in this research project?

You are being asked to participate in this study as a normal pregnancy (control group). We will compare the findings in normal pregnancies with those in higher risk pregnancies.

What will be my role in this Research Project?

The current standard of care at the Rotunda Hospital is that all pregnancies undergo a 20 week anatomy scan which includes an assessment of the fetal heart. Participating in this research project will involve one additional ultrasound scan of the baby's wellbeing and a more detailed examination of the fetal heart. The baby's heart will also be scanned after it is born.

However, before you decide whether or not to take part, it is important that you fully understand what the research is about and what you will be asked to do. It is important that you read the information below in order to make an informed decision and if you have any questions about any aspects of the study that are not clear to you, do not hesitate to ask me. Please make sure that you are satisfied before you decide to take part or not. It is important for you to know that deciding not to take part in this study will not affect your normal treatment in the Rotunda Hospital in any way

If you agree to participate you will be invited for an additional ultrasound scan of your baby's heart during your pregnancy (after 31 weeks of gestation). The scan will be undertaken in the fetal assessment unit (FAU) located on the second floor of the hospital. A qualified healthcare professional with expertise in maternal-fetal medicine and fetal and neonatal cardiology will perform your scan (the lead researcher and the principal investigators listed on this form).

Immediately following this, we will administer an oxygen test. This involves fitting you with a face mask which will supply oxygen for a total duration of ten minutes. Oxygen is present in the air we breathe and is safe to use in pregnancy. The reason we administer the oxygen through a face mask is to allow a higher flow rate (amount) to be given to you over a short period of time. The face mask is a plastic mask which will be fitted to cover over your nose and mouth. During this time you be lying down on the normal couch in the ultrasound department. Four stickers will be placed on your back to allow us to detect any changes in your own blood pressure or heart rate during the oxygen test. After ten minutes, a repeat ultrasound of the fetal heart will be performed. We will examine how the fetal heart has reacted to the oxygen test and this will hopefully allow us to understand better how that particular baby will adapt to newborn life and whether or not they will develop PPHN. The entire duration of your visit will be approximately 30 minutes.

As mentioned earlier a similar scan of your baby's heart will be performed on the baby after delivery. This will assess for the presence and severity of PPHN. All pregnancies will be followed up for outcomes at delivery.

Are there any Potential Harms/Risks?

Fetal and neonatal ultrasound is not associated with any identifiable maternal or fetal risks and there are no known risks to you or your baby when participating in this study.

Oxygen is safe to use in pregnancy, and high flow oxygen given for ten minutes duration is not known to be associated with any risks to you or your baby. Your own cardiovascular response will be monitored throughout the test. If for any reason you would like us to stop the test at any time we will do so.

In the rare event that the Fetal Medicine doctor performing your study examination identifies a problem during the study scan that could require a change in your pregnancy management (for example, evidence of deteriorating function of the placenta), she will alert your doctor/ medical team immediately, to coordinate on-going management plans for your pregnancy. Participation in this study will not affect the management of your pregnancy or the care of your baby, and responsibility for all clinical decisions in relation to your care and that of your baby will remain with your treating doctor and clinical team.

Are there any Potential Benefits?

Participation in this study is not likely to be associated with any specific benefit to you or your baby. However, you will receive an additional ultrasound scan which will assess fetal wellbeing and provide a more detailed cardiac assessment. Your baby will undergo a heart ultrasound when born which could potentially identify a cardiac problem at a very early stage.

What measures will be taken to ensure confidentiality?

We will respect completely your right to confidentiality. All data will be processed and analysed without being labelled with your name or medical history, and will be anonymized at the earliest opportunity. Regarding medical details and paper records obtained, these will be kept in a locked room and all electronic scan data will be stored on a single, password protected encrypted computer drive during the analysis of the data in the Rotunda Hospital. Data will be stored for a maximum of 5 years and will be destroyed confidentially after the study has been completed and published.

Other Considerations

We will require access to your medical records and those of your baby for the purpose of the research to collect data in relation to your pregnancy and its outcome and you are asked to consent to this information being collected and used for the purpose of this project. Your medical records will not leave the Rotunda Hospital and will be made available for you and your baby at each of your hospital visits.

Before you make a decision to participate or not, please read carefully the information provided and take the time to ask questions. If you wish to discuss any aspect of the study with any of the study researchers or with family and friends, or your GP please take the time to do so.

You may change your mind at any time and decide to withdraw before the start of the study or even after the study has commenced. You do not have to justify your decision to any person involved in the study. Your decision to participate or not in the research will have no effect on your care or the care of your baby in the hospital. If you have any further questions about the study, you may contact the lead researcher at the number below:

Dr Ann McHugh 01- 4022535.

At the completion of the project, will I be informed of the research outcome?

If you would like to know about the results of the research please let Dr Ann McHugh know. The results will be presented at scientific meetings and will be published in a medical journal but, again, we stress that no information will be released which would in any way identify you.

My rights as a Participant

1. I am informed that no information regarding my medical history will be divulged and the results of any tests involving myself will not be published so as to reveal my identity.
2. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to myself.
3. I understand that this research project has been approved by the relevant hospital Ethics Committee
4. I have been offered a copy of this document for my records

MATERNAL CONSENT FORM

To investigate the use of maternal hyper-oxygenation test to predict fetuses at risk of pulmonary hypertension.

Researchers: Prof. Fionnuala Breathnach
Dr Orla Franklin
Dr Afif El-Khuffash
Dr Ann McHugh

DECLARATION by participant: Please tick(✓) and provide your initials

1. I have read the information leaflet for this research study and I understand the contents. **Yes [ ] No [ ] initials [ ]**

2. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. **Yes [ ] No [ ] initials [ ]**

3. I fully understand that my participation is completely voluntary and that I am free to withdraw from the study at any time (prior to anonymisation/publication) without giving a reason and that this will not affect my care in any way. **Yes [ ] No [ ] initials [ ]**

4. I agree that my medical records and those of my baby will be accessible to the research team. The research team may need to access data relating to my clinical condition now, during my pregnancy and postpartum, my baby's measurements on ultrasound, my baby's clinical condition at birth and subsequently, and results of blood tests not included in the study. **Yes [ ] No [ ] initials [ ]**

One copy of this form must be retained by the participant and one copy must be retained by the researcher

5. I understand that information from this research will be published but that I will not be identified as a participant in this research in any publication.	**Yes [ ] No [ ] initials [ ]**
6. I understand that I will not be identified as a participant in this study (unless a legal requirement) and that the researchers may hold my anonymous information for 5 years after the study has been completed.	**Yes [ ] No [ ] initials [ ]**
7. I consent to my personal details being retained for a period of up to 5 years after this study has been completed and used to invite me to participate in future research in accordance with this consent.	**Yes [ ] No [ ] initials [ ]**
8. I agree that an echocardiogram can be performed on my baby after it is born as part of this study.	**Yes [ ] No [ ] initials [ ]**
9. I consent to future use of my anonymised data in follow-on studies, without further consent, where ethics committee approval has been given.	**Yes [ ] No [ ] initials [ ]**
10. I understand that the researchers undertaking this research will hold in confidence and securely all collected data and other relevant information.	**Yes [ ] No [ ] initials [ ]**
11. I freely and voluntarily consent to participating and allowing my baby to participate in this research study.	**Yes [ ] No [ ] initials [ ]**

PARTICIPANT'S NAME ……..

Contact Address……

……

Phone number……..Email:……..

Participant's signature:……..Date:……..

Name of person taking consent:……Signature:……....Date:……

Researcher:…….....Signature:……Date:…….

One copy of this form must be retained by the participant and one copy must be retained by the researcher

Participation Information Leaflet

Thank you for taking the time to read this Information Leaflet

Title of the Research Project

Can sonographic assessment of pulmonary vascular reactivity following the maternal hyperoxygenation test predict neonatal outcome in fetuses at risk of pulmonary hypertension.

Who are the Researchers?

Lead Researcher: Dr Ann McHugh, SpR Obstetrics and Gynaecology, Rotunda Hospital **Telephone No: 01- 4022535.**

Principal Investigators: Professor Fionnuala Breathnach, Obstetrics and Gynaecology, Rotunda Hospital – RCSI unit. Dr. Orla Franklin, Neonatal cardiology, Our Lady's Hospital for Sick Children, Dublin/ The Rotunda Hospital, Dublin. Dr Afif El-Khuffash, Clinical Director, Neonatology, Rotunda Hospital

What is this Research Project about?

We are investigating a medical condition called **P**ersistent **P**ulmonary **H**ypertension of the **N**ewborn (PPHN). This is a medical problem that can develop after the baby is born and while in the newborn period. The condition can lead to serious respiratory (breathing) and cardiovascular (heart) problems in the newborn.

Recent studies have shown that there is a test that can be performed during pregnancy to predict which newborns are likely to develop the condition and which newborns will develop a severe form of PPHN. By examining how the fetal heart (the 4 pumping chambers of the heart and the vessels coming out of the heart that supply the body) reacts in the womb to oxygen given to the mother, we may be able to predict more accurately which babies will develop PPHN and which of those will have a severe form of the condition.

One copy of this form must be retained by the participant and one copy must be retained by the researcher

PPHN occurs in up to 7 per 1000 live births when the normal circulatory (heart) transition in the early newborn period fails to occur. If we knew before delivery which babies would suffer with this condition, it would allow us put in place the most effective management plan for both mother and baby.

Why am I being asked to participate in this research project?

You are being asked to participate in this study as a non-pregnant control. We will compare the findings in non maternal subjects with normal pregnancies and with those in higher risk pregnancies.

What will be my role in this Research Project?

Before you decide whether or not to take part, it is important that you fully understand what the research is about and what you will be asked to do. It is important that you read the information below in order to make an informed decision and if you have any questions about any aspects of the study that are not clear to you, do not hesitate to ask me. Please make sure that you are satisfied before you decide to take part or not. It is important for you to know that deciding not to take part in this study will not affect any future treatment in the Rotunda Hospital in any way

If you agree to participate you will be administered an oxygen test. This involves fitting you with a face mask which will supply oxygen for a total duration of ten minutes. Oxygen is present in the air we breathe and is safe to use in pregnancy. The reason we administer the oxygen through a face mask is to allow a higher flow rate (amount) to be given to you over a short period of time. The face mask is a plastic mask which will be fitted to cover over your nose and mouth. During this time you be lying down on the normal couch in the ultrasound department. Four stickers will be placed on your back to allow us to detect any changes in your own heart rate during the oxygen test. The entire duration of your visit will be approximately 30 minutes.

Are there any Potential Harms/Risks?

Oxygen is safe to use and high flow oxygen given for ten minutes duration is not known to be associated with any risks to you.Your own cardiovascular response will be monitored throughout the test. If for any reason you would like us to stop the test at any time we will do so.

Are there any Potential Benefits?

Participation in this study is not likely to be associated with any specific benefit to you.

What measures will be taken to ensure confidentiality?

We will respect completely your right to confidentiality. All data will be processed and analysed without being labelled with your name or medical history, and will be anonymized at the earliest opportunity. Regarding medical details and paper records obtained, these will be kept in a locked room and all electronic scan data will be stored on a single, password protected encrypted computer drive during the analysis of the data in the Rotunda Hospital. Data will be stored for a maximum of 5 years and will be destroyed confidentially after the study has been completed and published.

Other Considerations

We will require access to your medical records (if applicable) for the purpose of the research and you are asked to consent to this information being collected and used for the purpose of this project. Your medical records will not leave the Rotunda Hospital.

Before you make a decision to participate or not, please read carefully the information provided and take the time to ask questions. If you wish to discuss any aspect of the study with any of the study researchers or with family and friends, or your GP please take the time to do so.

You may change your mind at any time and decide to withdraw before the start of the study or even after the study has commenced. You do not have to justify your decision to any person involved in the study. Your decision to participate or not in the research will have no effect on your future care in the hospital. If you have any further questions about the study, you may contact the lead researcher at the number below:

Dr Ann McHugh 01- 4022535.

At the completion of the project, will I be informed of the research outcome?

If you would like to know about the results of the research please let Dr Ann McHugh know. The results will be presented at scientific meetings and will be published in a medical journal but, again, we stress that no information will be released which would in any way identify you.

My rights as a Participant

1. I am informed that no information regarding my medical history will be divulged and the results of any tests involving myself will not be published so as to reveal my identity.
2. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to myself.
3. I understand that this research project has been approved by the relevant hospital Ethics Committee
4. I have been offered a copy of this document for my records

CONSENT FORM

To investigate the use of maternal hyper-oxygenation test to predict fetuses at risk of pulmonary hypertension.

Researchers: Prof. Fionnuala Breathnach
Dr Orla Franklin
Dr Afif El-Khuffash
Dr Ann McHugh

DECLARATION by participant: Please tick(□) and provide your initials

1. I have read the information leaflet for this research study and I understand the contents.	**Yes [ ] No [ ] initials [ ]**
2. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction.	**Yes [ ] No [ ] initials [ ]**
3. I fully understand that my participation is completely voluntary and that I am free to withdraw from the study at any time (prior to anonymisation/publication) without giving a reason and that this will not affect my care in any way.	**Yes [ ] No [ ] initials [ ]**
5. I understand that information from this research will be published but that I will not be identified as a participant in this research in any publication.	**Yes [ ] No [ ] initials [ ]**
6. I understand that I will not be identified as a participant in this study (unless a legal requirement) and that the researchers may hold my anonymous information for 5 years after the study has been completed.	**Yes [ ] No [ ] initials [ ]** **Yes [ ] No [ ] initials [ ]**
7. I consent to my personal details being retained for a period of up to 5 years after this study has been completed and used to invite me to participate in future research in accordance with this consent.	**Yes [ ] No [ ] initials [ ]**
8. I consent to future use of my anonymised data in follow-on studies, without further consent,	**Yes [ ] No [ ] initials [ ]**

where ethics committee approval has been given.

Yes [ ] No [ ] initials [ ]

9. I understand that the researchers undertaking this research will hold in confidence and securely all collected data and other relevant information.

Yes [ ] No [ ] initials [ ]

10. I freely and voluntarily consent to participating in this research study.

Yes [ ] No [ ] initials [ ]

PARTICIPANT'S NAME ……..
Contact Address/email address
……
……
Participant's signature:……..Date:……..

Name of person taking consent:……Signature:……....Date:……
Researcher:…….....Signature:……Date:…….

One copy of this form must be retained by the participant and one copy must be retained by the researcher

HPRA
An tÚdarás Rialála Táirgí Sláinte
Health Products Regulatory Authority

Clinical Trial Protocol Template

1 INFORMATION ON CLINICAL TRIAL PROTOCOL TEMPLATE

This protocol template has been designed for clinical trials which are subject to the European Communities (Clinical trials on Medicinal Products for Human Use) Regulations, 2004 (S.I. No 190 of 2004), as amended. The template is available for use by all investigators who are carrying out clinical trials if they so wish, however there is no requirement to use it.

All advisory text and quotations from GCP are in parentheses i.e. < >. All of these should be deleted before finalising the document.

All sample text is in 'basic text' style. This text should be altered or deleted as required while the draft is being developed.

2 STUDY TITLE

Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension?

3 STUDY SPONSOR

Royal College of Surgeons in Ireland

4 APPLICATION DETAILS

4.1 Study title

Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension?

Persistent pulmonary hypertension of the newborn (PPHN) is a relatively common condition occurring in 0.5 to 7 per 1000 live births and can result in significant cardiovascular instability in the newborn. It occurs when there is a failure of the normal circulatory transition in the early newborn period. Persistence of the fetal circulation occurs, resulting in pulmonary hypertension, low oxygen levels and marked right-to-left shunting of blood in the newborn heart. It results in a mortality ranging between 4 to 33% [1-2]. Some degree of pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure. Increased pulmonary vascular resistance in the newborn will produce extrapulmonary shunting of blood which can lead to severe and potentially unresponsive hypoxemia and

significant morbidity and mortality. Pulmonary hypertension typically accompanies pulmonary hypoplasia when diminished surface area for gas exchange and inadequate pulmonary blood flow lead to hypoxia and remodelling of the resistant pulmonary arterioles [3]. Pulmonary hypoplasia results in parenchymal lung disease and pulmonary vascular disease resulting in pulmonary arterial hypertension The ability to predict the occurrence and severity of neonatal pulmonary hypertension antenatally, would facilitate optimal antenatal and neonatal management.

In the human fetus, blood flow velocity waveforms can be recorded at all cardiac levels, including at the foramen ovale, atrioventricular valves, outflow tracts, pulmonary arteries and ductus arteriosus [3]. Velocity waveforms may be recorded from the right and left pulmonary arteries or from peripheral vessels within the lung. Analysis of the waveforms using ultrasound Doppler can be used to study the normal development of fetal lung circulation [4]. Doppler examination of blood flow in the main stem of both the right and the left pulmonary arteries of the fetus is feasible, and increases our insight into the lung perfusion of the human fetus.

Recent studies have shown that fetal pulmonary vasculature reacts to maternal hyperoxygenation [5-7]. Following maternal oxygen therapy, a decrease in the pulmonary vascular resistance as demonstrated by pulmonary venous Doppler, is deemed to indicate vasoreactivity in the pulmonary vascular bed [8]. Small studies to date indicate that a lack of vasoreactivity in response to maternal hyperoxygenation, may serve as a useful clinical tool in predicting lethal pulmonary hypoplasia in those at- risk fetuses. The measurement of peripheral pulmonary velocity waveforms before and after maternal hyperoxygenation may therefore help in determining the risk of developing PPHN.

We will perform a feasibility cohort study. Data will be collected prospectively. It will evaluate the use of fetal echocardiographic Doppler assessment of the pulmonary vasculature prior to and following maternal hyperoxygenation therapy to predict fetuses at risk of pulmonary hypertension in the neonatal period. The study will be undertaken in the Rotunda Hospital, Dublin.

The primary study objective is to predict the occurrence of neonatal pulmonary hypertension by measuring the pulmonary artery reactivity to maternal hyperoxygenation in fetuses at risk of neonatal respiratory morbidity. We aim to assess the vasoreactive response to maternal hyperoxygenation in utero. We aim to evaluate the ability of the maternal hyperoxygenation test to predict the degree of pulmonary vasculopathy present prior to birth. Additionally, our objective is to evaluate if pulmonary artery (PA) reactivity to maternal hyperoxygenation identifies fetuses that will require urgent neonatal intervention. While the test is being conducted we will also assess the changes in maternal haemodynamics by measuring serial changes in maternal cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR) before, during and after maternal hyperoxygenation.

Traditionally, maternal haemodynamic evaluation relied on invasive methods such as pulmonary artery catheterization, or on less invasive, but technically demanding and time consuming echocardiographic assessment. Development of non-invasive cardiac output monitoring (NICOM) based on transthoracic bio-reactance has now provided the possibility to investigate maternal haemodynamic changes during pregnancy on a much larger scale. The non-invasive cardiac output measurement (NICOM®) system has acceptable accuracy, precision and responsiveness for cardiac output monitoring in patients in a wide range of

circulatory situations and outputs correlate accurately with those obtained from invasive haemodynamic assessments [9]. Using this non-invasive approach we can evaluate the changes in the maternal haemodynamics during the period of hyper oxygenation.

4.2 Reference numbers

Protocol identification (code or reference number): HOTPOT1
EudraCT number: 2016-003181-12
Date and version number: 28/07/2016 V01

4.3 Applicant details

Chief investigator/ Co-ordinating investigator:

Prof. Fionnuala Breathnach
Associate Professor Obstetrics and Gynaecology
Maternal Fetal Medicine Specialist
Royal College of Surgeons in Ireland
Rotunda Hospital
Parnell Square
Dublin 1
Tel: +353 14022540
Mob: +353 868213744
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Principal Investigators:

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Specialist Registrar in Obstetrics and Gynaecology
Royal College of Surgeons in Ireland
Master's House
Rotunda Hospital
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Dr Orla Franklin,
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Dr Afif EL-Khuffash FRCPI, MD, DCE
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Honorary Clinical Senior Lecturer
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Funder:
Friends of the Rotunda
Pillar Room
Rotunda Hospital
Parnell Street
Dublin 2
Tel: (01) 872 2377

4.4 Signatures

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Associate Professor Obstetrics and Gynaecology
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Date:

Dr Ann McHugh
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Date:

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5 CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

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7 DOCUMENT HISTORY

Document	Date of Issue	Summary of Change
Protocol V02	14/09/2016	Changes as advised by HPRA
Original protocol V01	28/07/2016	Not applicable

8 SYNOPSIS

Title of study	Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension?
Name of sponsor/company	Royal College of Surgeons in Ireland
Objectives	The main objectives of this feasibility study are as follows: 1. Assess vasoreactive response to maternal hyperoxygenation (MH) in utero. 2. Assess the ability of MH to predict the degree of pulmonary vasculopathy present prior to birth. 3. Evaluate if pulmonary artery (PA) reactivity to MH identifies fetuses that will develop pulmonary hypertension. 4. Predict neonatal survival and pulmonary hypertension by measurement of PA reactivity to MH in fetuses at risk of neonatal respiratory morbidity. 5. Assess serial changes in maternal haemodynamics (cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR)) before, during and after MH.

Trial design	This is a feasibility study. All data will be collected prospectively. It will evaluate the use of fetal echocardiographic Doppler assessment of the pulmonary vasculature prior to and following maternal hyperoxygenation therapy to predict the development of pulmonary hypertension in the neonatal period. The presence of pulmonary hypertension in the neonate will be formally assessed with a neonatal echocardiogram performed at two separate time points, at 6-12 hours of life and at 36-48 hours of life. Persistent pulmonary hypertension will be defined by echocardiography as well as by clinical indicators as follows. 1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and, 2) Normal Structural anatomy of the heart on echocardiogram; and, 3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or 4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic 5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity. The Study will be undertaken in the Rotunda Hospital, Dublin.
Key inclusion criteria	The inclusion criteria can be divided into 4 main categories: **A) <u>Those at risk of respiratory morbidity at term</u>** • Iatrogenic elective Caesarean section being performed < 38 gestational weeks in an otherwise well baby. This subgroup will be informative in

	relation to circulatory adaptation close to term. **B) <u>Those at risk of pulmonary hypoplasia</u>** • Patients with mid trimester PPROM • Patients with persistent oligohydramnios of renal or nonrenal origin • Patients whose fetuses have known: Congenital diaphragmatic hernia (CDH), Congenital cystic adenomatoid malformation (CCAM) • Other space occupying lesions of the thorax (cardiomegaly, pleural effusion and skeletal dysplasia). **C) <u>Those at risk of respiratory morbidity due to a cardiac cause</u>** • Women whose fetuses have a prenatal diagnosis of moderate/severe perimembranous ventricular septal defect (VSD)/atrioventricular septal defect (AVSD) in the absence of other structural heart disease including cases of Trisomy 21. The MH test may contribute to prediction of the need for neonatal intervention in this group. D) **<u>Normal pregnancy control group</u>**
Key exclusion criteria	The exclusion criteria are as follows: • Maternal age < 18 years • Known fetal chromosomal abnormality excluding Trisomy 21 • Gestational age <31 weeks and >40 weeks • Maternal chronic respiratory disease (including COPD, Cystic Fibrosis, Pulmonary Fibrosis) • Maternal congenital heart disease • Maternal use of bleomycin or

	amiodarone • Current maternal use of nitrofurantoin or use within the last 7 days. The justification for the exclusion criteria are as follows: • An adult is defined as a person aged 18 years or over. • The literature indicates that maternal hyperoxygenation does not alter fetal pulmonary circulation until after 31 weeks and therefore we will perform the test after 31 weeks gestation. • The use of high flow oxygen is contraindicated in patients with chronic respiratory disease. • Pregnant women with congenital heart disease are high risk antenatal patients and will be excluded to avoid them being exposed to high flow oxygen and to avoid any misinterpretation of the NICOM. • High flow oxygen should be avoided in any person taking the medications bleomycin or amiodarone and nitrofurantoin.
Number of subjects	This is a feasibility study; therefore the number of subjects to be studied will be 60-75 in total. N=60 (+/- 15 subjects) This will be divided as 15 subjects per group. In group D- the normal pregnancy controls. Subjects may be recruited to this group however may eventually end up in group A (if they require a caesarean section < 38 weeks gestation) or in group B (if they develop persistent oligohydramnios after recruitment. For this reason 15-30 subjects will be recruited to group D to allow for a drop out in this group.
Test product, dose and mode of administration	The drug is medical oxygen, (O2 100% v/v inhalational gas). It will be administered to the subject via a partial rebreather face mask

	at a rate of 8-10 litres per minute for a duration of ten minutes with the patient lying in a semi recumbent position. Maternal haemodynamics will be monitored non-invasively before, during and after the administration of the oxygen using the NICOM (non- invasive cardiac output monitoring) system.
Duration of treatment	The duration of treatment with high flow oxygen is 10 minutes in total. The study procedure will take 30 minutes in total.
Statistical methods	Given that this is a pilot study and small numbers of subjects will be recruited, descriptive statistics will be used to summarize the findings. We hope that this study would inform a larger study or randomised control trial in the future.
Sample size	60 to 75 subjects in total

9 ABBREVIATIONS

AAS	Atrial septal aneurysm
AC	Abdominal circumference
AFI	Amniotic fluid index
AT	Acceleration time
AVSD	Atrioventricular Septal Defect
BPD	Biparietal diameter
cAMP	Cyclic adenosine monophosphate
CCAM	Congenital cystic adenomatoid malformation
CDH	Congenital diaphragmatic hernia
cGMP	Cyclic guanyl monophosphate
CMH	Chronic maternal hyperoxygenation
CO	Cardiac output
DA	Ductus arteriosus
DV	Ductus venosus
ECMO	Extracorporeal membrane oxygenation
ET	Ejection time
ET-pCO2	Adjusted end tidal partial pressure of carbon dioxide
FBM	Fetal breathing movements
FGM	Fetal generalised movements
FHR	Fetal heart rate
FiO2	Fraction of inspired oxygen
FL	Femur length
HC	Head circumference
HFOV	High frequency oscillatory ventilation
iNO	Inhaled nitric oxide

IUGR	Intrauterine growth restriction
MAP	Mean arterial pressure
MDT	Multidisciplinary team meeting
MH	Maternal Hyperoxygenation
NICOM	Non-Invasive Cardiac Output Monitoring
NICU	Neonatal intensive care unit
NO	Nitric oxide
PA	Pulmonary artery
pCO2	Partial pressure of carbon dioxide
PDA	Patent ductus arteriosus
PEEP	Positive end expiratory pressure
PFO	Patent foramen ovale
pH	Potential hydrogen
PI	Pulsatility Index
PIP	Peak inspiratory pressure
PPHN	Persistent pulmonary hypertension of the neonate
PPROM	Preterm prelabour rupture of membranes
PVR	Pulmonary vascular resistance
PW	Pulsed wave
SV	Stroke volume
SVR	Systemic vascular resistance
TVI	Time velocity integral
UA	Umbilical artery
VSD	Ventricular Septal Defect

10 INTRODUCTION

10.1 Background information

Pulmonary hypoplasia is defined as a decrease in the size and volume of the lungs because of a reduction in the number of cells, airways and alveoli [10]. It causes severe neonatal respiratory compromise, with a substantial risk for neonatal mortality. Several conditions identifiable in utero can result in pulmonary hypoplasia, including congenital diaphragmatic hernia, fetal lung malformations and skeletal dysplasia. Pulmonary hypoplasia has been well documented in fetuses with long standing oligohydramnios, which may be a result of chronic amniotic fluid leakage or fetal renal disease. The extent of fetal pulmonary hypoplasia depends on the degree and the duration of the oligohydramnios and on the gestational age and the stage of lung development when it occurs. The prenatal prediction of pulmonary hypoplasia is important for perinatal management and parental counselling, particularly in cases that will require intensive respiratory therapy immediately after birth. Accurate prenatal prediction of lethal pulmonary hypoplasia further informs the perinatal management of such cases, with, for example, appropriate decision-making on site and mode of delivery.

Several methods have been proposed to assess fetal pulmonary vascular development including measurement of fetal chest circumference, chest area, chest area minus heart area, ratio of chest circumference to abdominal circumference, ratio of chest area to heart area and ratio of the chest area minus the heart area to the chest area [11]. Some of these methods are time

consuming to perform and there can be considerable variability in measurements between different ultrasonographers. The use of chest circumference requires an accurate knowledge of the length of gestation; therefore it cannot be used in fetuses with an unknown gestational age or fetuses with suspected intrauterine growth restriction. The chest circumference/abdominal circumference ratio cannot be used in fetuses with large chest circumferences, therefore fetuses with polycystic kidneys, obstructive uropathy or omphalocele would be excluded. Measuring the chest circumference and chest circumferences/abdominal circumference ratio has not been investigated in cases of prolonged rupture of membranes which make up many cases of pulmonary hypoplasia [12]. The ability to accurately predict the occurrence and severity of pulmonary hypertension by a method that is non-invasive and reproducible would be extremely beneficial in both obstetric management and in parental counseling.

Recent studies have shown that fetal pulmonary vasculature reacts to maternal hyperoxygenation [5-7]. Following maternal oxygen therapy, a decrease in the pulmonary vascular resistance as demonstrated by pulmonary arterial Doppler, is deemed to indicate vasoreactivity in the pulmonary vascular bed. This is mainly caused by a release of endothelium-derived nitric oxide, which leads to vasodilatation of the pulmonary arterial bed. Small studies to date indicate that a lack of vasoreactivity in response to maternal hyperoxygenation may serve as a useful clinical tool in predicting pulmonary hypoplasia in those at- risk fetuses [13].

We believe there will be a significant decrease in the pulsatility index in both proximal pulmonary arteries in fetuses that will develop PPHN; suggesting a significant decrease in pulmonary vascular impedance similar to that as reported by Rasanen et al [14]. Those investigators demonstrated that the pulsatility indices in the distal and proximal pulmonary arteries decrease during the second half of pregnancy, which reflects the total blood flow and vascular impedance in the lung. On the basis of these early findings, blood flow waveforms from the proximal pulmonary arteries will be measured in this study.

In addition, babies born by elective caesarean section prior to 38 weeks gestation for either maternal or fetal indications are at increased risk of neonatal pulmonary hypertension. Tita et al noticed that elective repeat cesareans before 39 weeks of gestation are common and are associated with respiratory and other adverse neonatal outcomes [15]. Hourani et al found that there were significantly higher risks in the early Cesarean section group (37+0-37+6 weeks gestation) compared with caesarean section after 38 weeks, of various neonatal outcomes including respiratory complications, hypothermia and feeding difficulty. In the early Cesareans group, the risk for respiratory complications was 5.8 times higher (P=0.0001) manifested most often as transient tachypnea of the newborn and this risk decreased with advancing gestational age [16]. Evaluation of this subgroup will be informative in relation to fetal circulatory adaptation close to term.

The presence of pulmonary hypertension in the neonate will be formally assessed with a neonatal echocardiogram performed at two separate time points, at 6-12hours of life and at 36-48 hours of life. Persistent pulmonary hypertension will be defined by echocardiography as well as by clinical indicators as follows:
1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,
2) Normal Structural anatomy of the heart on echocardiogram; and,

3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or
4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic
5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity.

The novelty and impact of this study is that this research has never been conducted in Ireland. The hyperoxygenation test has been used internationally in a number of different settings including in the assessment of growth restricted babies and in those with many cardiac conditions Persistent pulmonary hypertension of the newborn (PPHN) is a relatively common condition occurring in 0.5 to 7 per 1000 live births and results in a mortality ranging between, 4% to 33% [1-2]. Inhaled nitric oxide (iNO) and extracorporeal membrane oxygenation (ECMO) are the only current therapeutic options that are systematically evaluated in clinical trials [17]. The vasodilatation induced by iNO is mediated by increasing concentrations of the second messengers: cyclic guanyl monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) in pulmonary vascular smooth muscle. The widespread use of iNO has resulted in a reduction in the need for ECMO; however, mortality and long-term morbidity remain unchanged [1]. In addition, up to 40% of infants treated with iNO either have a transient response or fail to demonstrate an improvement in oxygenation [18]. iNO does not improve myocardial performance in infants with PPHN, which often accompanies the condition and may contribute to mortality. Furthermore, the increasing cost of administering iNO to infants with PPHN may prohibit its use in developing countries. The condition is clinically characterised by hypoxemic respiratory failure due to failure of adequate transition of the pulmonary vasculature from a high resistance fetal to a low resistance extra uterine circuit. Pulmonary vascular resistance (PVR) remains high resulting in right to left shunting across the patent foramen ovale (PFO) and the patent ductus arteriosus (PDA) resulting in hypoxemia. Despite recent advances in the management of PPHN, the risk of mortality and adverse neurological sequelae remains high. By undertaking this study we may be able to identify a way of accurately predicting the outcomes in babies at risk of PPHN. The study is a feasibility study which may lead to larger scale studies in the future. These studies may impact our clinical management of pregnancies where the fetus is at risk of developing PPHN. The study may also impact our routine care of babies at risk of PPHN, in that; they may all undergo an echocardiogram and maternal hyperoxygenation test in the future. This may help in identifying those fetuses with a poorer neonatal outcome.

Study population:

Women who are carrying a fetus at risk of pulmonary hypoplasia will be identified through the hospital records system (Current Inpatients, ultrasound department [anatomy scans], fetal medicine multidisciplinary team meeting [MDT] meetings), and will be offered participation in the study as part of a comprehensive fetal echocardiography. Pregnant women attending for scheduled caesarean sections prior to 38 weeks gestation will also be recruited to the study.

Fifteen to thirty women with normal pregnancies will also be recruited to the study to function as the control group. These women will be identified at their routine anatomy scan appointment in the hospital between 20-22 weeks of gestational age and invited to participate in the study. If they are agreeable they will be asked to return after 31 weeks gestation to participate in the study.

10.2 Rationale for the study

Study Rationale:

Fetal circulation is unique. The placenta, instead of the lung, functions as the organ for gaseous exchange. Pulmonary hypertension is a normal state for the fetus and pulmonary vascular tone increases with increasing gestational age. In the fetus, the right ventricular output crosses the ductus arteriosus to the aorta, and only 5-10% of the combined ventricular output is directed to the pulmonary vascular bed [19]. This low oxygen tension environment that promotes high intrinsic myogenic tone and high vasocontractility, changes dramatically at birth. There is a reduction in pulmonary arterial pressure and resistance due to an increase in oxygen tension and a 10-fold rise in pulmonary blood flow [20]. Persistent pulmonary hypertension of the newborn occurs when this normal transition from fetal to neonatal life fails to occur.

Oxygen is a colourless, odourless gas which is present in the atmosphere at 21%. Oxygen may be administered at concentrations of up to 100%; however, with most medical delivery systems the actual inspired concentration will rarely exceed 60%. High flow oxygen therapy, with concentrations up to 60% for short periods of time is safe. In this setting, it is also safe to administer to patients with severe asthma, pulmonary embolism, pneumonia and fibrosing alveolitis [21]. Lower oxygen concentrations should be administered to patients which chronic obstructive airway disease, as physiologically, they have a hypoxic drive for respiration. In the presence of high levels of oxygen patients with chronic obstructive airway disease will under-ventilate their lungs leading to a respiratory acidosis and respiratory arrest in severe cases. The pharmacology, pharmokinetics and toxicology of oxygen are well-known.

Potential adverse effects of supplemental oxygen therapy include parenchymal lung injury, airway injury and absorptive atelectasis, however, these complications of oxygen toxicity are reported in cases of long-term oxygen therapy where reactive oxygen intermediates can promote inflammation and induce cell death. These adverse effects have not been demonstrated with the use of short duration oxygen therapy. Exposures of up to 60% for up to one week have not been proven to cause any specific lung injury. High flow concentrations of oxygen (>60%) may damage the alveolar membrane when inhaled for more than 48 hours [22]. Administration of 100% oxygen for more than 24-30 hours will result in substernal chest pain and mild dyspnoea.

The only contraindication for the use of oxygen is in the presence of a naked flame and with active smoking, as it supports combustion. Facial burns and death of patients who smoke when using oxygen are well documented. There are no contraindications for oxygen therapy during

pregnancy and breast-feeding [21]. Interactions with other medicinal products include amiodarone and bleomycin-induced lung disease.

Physiologically in pregnancy, there is a significant increase in the demand for oxygen owing to the increased metabolic rate and a 20% increased consumption of oxygen. Resting minute ventilation and tidal volume increase as does arterial pO2. A mild fully compensated respiratory alkalosis is normal in pregnancy. Oxygen therapy has been investigated in pregnant patients in a multitude of settings including for fetal distress in labour.

In a study of 15 mothers, a mixture of room air and oxygen (100% at 9L/min) was administered via a face mask for at least 10 minutes. The authors concluded that they did not observe any adverse maternal or fetal side effects during or immediately after the oxygen administration [23].

The following study examined 407 patients over a 5-year period, of which 35 received long term maternal hyperoxygenation for the investigation of severe early onset IUGR. The mothers were hospitalized, rested in bed and given humidified oxygen to breath via a medium concentration face mask at a rate of 8L/min (delivering about 55% of oxygen) continuously, apart from the daily needs of hygiene and alimentation. In two of the 35 patients undergoing MH, therapy was interrupted for 2 days because of maternal hyperemesis. There were no other maternal complications [24].

Eight pregnant women were investigated in another study and the authors described a decrease in adjusted end tidal pCO2 (ET-pCO2) by a mean of 12% from baseline while 100% oxygen was administered, this returned back to baseline during normocapnic hyperoxygenation. Minute ventilation increased by an average of 13%. Maternal oxygen saturation did not change significantly throughout the different stages of the study which included administration of 100% oxygen for 10-15minutes [7].

The effect of MH on left heart filling in fetuses with atrial septal aneurysm (AAS) causing impediment to left ventricular inflow was investigated. The authors performed fetal echocardiography prior to and at 10 minutes of MH in 12 fetuses with AAS and concluded that short term MH increases fetal pulmonary venous return , substantially alters left ventricle geometry and promotes antegrade flow in the aortic isthmus. This demonstrates proof of concept that MH can improve filling of the left side of the fetal heart in AAS. They found short term administration of MH safe, with no negative effects noted. MH has now become a standard diagnostic tool used in echocardiographic assessment of all fetuses with hypoplastic left heart at their institution in Philadelphia [25].

A study of 9 pregnant patients was conducted where the patients had oxygen administered for 10 minutes of acute MH at 8L/min, 100% FiO2 via a non-rebreather face mask. The authors also evaluated intermittent chronic MH (CMH) therapy consisting of >8 hours a day of oxygen at 8-9 L/min of 100% FiO2 at 26 weeks of gestation until delivery, for its effect on aortic and mitral valve annular dimensions in fetuses with left heart hypoplasia. They found no significant maternal or fetal complications in the CMH cohort. One mother on CMH developed epistaxis on the first day of therapy, which resolved with the addition of a humidifier. There was no evidence of retinopathy on ophthalmological exam. They concluded that there were no significant maternal or fetal complications of acute or chronic maternal hyperoxygenation use [26].

In another study of 25 singleton pregnancies, where the mothers received 100% oxygen at a flow rate of 8-10L/min, the authors could not demonstrate any haemodynamic changes that suggested adverse effects related to oxygen administration [27]. These findings echo those of others who have not demonstrated any deleterious effects on the fetus during maternal administration of 100% oxygen for periods of about 60-70 minutes [28-29].

The authors of a study of 15 patients who were administered chronic intermittent materno-fetal hyperoxygenation in late gestation concluded that given its simplicity, universal availability and potential benefits for large numbers of patients, intensive research at dedicated centres is now required with the goal to optimize hyperoxygenation schedules, define all suitable cardiac subjects and assess short and long term safety of this novel therapeutic approach [30].

Short term administration of extra oxygen to pregnant women with pregnancies complicated by intrauterine growth restriction (IUGR) has been reported to give rise to a transient increase in fetal generalised movements (FGM) and in fetal breathing movements (FBM) and in fetal heart rate variation (FHR). The increase of the PI from intracerebral vessels and the decrease of the PI from the descending aorta, and the suggested restoration of right ventricular dominance during short term hyperoxygenation indicate that the haemodynamics of the IUGR fetus can be affected as a result of MH [13].

The use of oxygen during pregnancy should follow the same general principles as the use of oxygen for other patients. Oxygen is commonly given as part of the treatment for many obstetric emergencies. However, when oxygen is administered during pregnancy or labour, clinicians should aim to achieve normoxaemia (saturation 94–98%). There is no randomised trial evidence to suggest that maternal ''hyperoxaemia'' is beneficial to mother or fetus. A Cochrane review of the use of supplemental oxygen for caesarean section during regional analgesia concluded as follows: "Current evidence suggests that supplementary oxygen given to healthy term pregnant women during elective caesarean section under regional anaesthesia is associated with higher maternal and neonatal oxygen levels (maternal SpO2, PaO2, UaPO2 and UvPO2) and higher levels of oxygen free radicals. However, the intervention was neither beneficial nor harmful to the neonate's short-term clinical outcome as assessed by Apgar scores [31].

Although maternal oxygen therapy can increase fetal oxygen levels, there are limited data regarding its risks or possible benefits to the mother, fetus, and newborn. Women who suffer from major trauma, sepsis or acute illness during pregnancy should receive the same oxygen therapy as any other seriously ill patients, with a target oxygen saturation of 94–98%. The same target range should be applied to women with hypoxaemia due to acute complications of pregnancy (e.g., collapse related to amniotic fluid embolus, eclampsia or antepartum or postpartum haemorrhage). Women with underlying hypoxemic conditions (e.g. heart failure) should be given supplemental oxygen during labour to achieve an oxygen saturation of 94–98% unless they are at risk of hypercapnic respiratory failure (target range 88-92%) [32]. This study illustrates how oxygen can be used safely in a number of different ways during a pregnancy.

In our study administration of oxygen will be by a partial rebreathing oxygen mask, which is a simple mask attached to a reservoir. Oxygen concentration from 50-60% can be achieved with oxygen flow rates between 10 and 12 L/min. With most delivery systems inspired concentrations over 60% are unlikely to be achieved.

11 STUDY OBJECTIVE

Study objective:
1. Assess vasoreactive response to maternal hyperoxygenation (MH) in utero.
2. Assess the ability of MH to predict the degree of pulmonary vasculopathy present prior to birth.
3. Evaluate if pulmonary artery (PA) reactivity to MH identifies fetuses that will develop PPHN.
4. Predict neonatal survival and pulmonary hypertension by measurement of PA reactivity to MH in fetuses at risk of neonatal respiratory morbidity.
5. Assess the impact of MH on maternal haemodynamics by assessing serial changes in cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR) before, during and after MH.

11.1 Primary objective

The overall aim of the study is to assess the ability of the hyperoxygenation test to predict the presence of persistent pulmonary hypertension of the newborn in fetuses at risk of this condition.

The primary outcome of interest is the occurrence pulmonary hypertension in the neonate measured using echocardiography on Day 1 and 2 of age.

This pilot study will assess feasibility of recruitment and acceptability of the hyperoxygenation test in pregnant women. The results of this study will help inform a larger multicentre definitive intervention study.

11.2 Secondary objective

The secondary objective of the study is to assess for the following secondary outcomes.

Secondary outcomes
Severity of pulmonary hypertension
Composite of neonatal respiratory morbidity (Respiratory Distress Syndrome and Transient Tachypnea of the Newborn)
Neonatal intensive care unit (NICU) admission
28 day survival
Survival to discharge from hospital
Meconium Aspiration Syndrome
Umbilical Artery and vein pHs and base excess
Neonatal interventions required:

- Chest compressions
- Intubation
- Peak inspiratory pressure (PIP)
- Mean arterial pressure (MAP)
- Positive end expiratory pressure (PEEP)

- Adrenaline bolus at birth
- Hydrocortisone pre transfer
- Inotrope infusion pre transfer
- Nitric
- Surfactant
- High frequency oscillatory ventilation (HFOV) at birth
- Intravenous antibiotics
- Nitric oxide duration

11.3 Exploratory objectives

N/A

11.4 Primary and secondary/exploratory endpoints/outcome measures

Doppler echocardiography will be performed on fetuses between 31-40 weeks gestation. A fetal echocardiogram will be performed according to an agreed protocol. Image-directed pulsed and colour Doppler equipment (Volusson E8) with a 5MHz sector probe will be used to obtain blood velocity waveforms. The lowest high-pass filter level (100Hz) will be used and the spatial peak temporal average power output for colour and pulsed Doppler kept at <100mW/cm. An angle of < 15 degrees between the vessel and Doppler beam as assessed by colour Doppler will be accepted for analysis. A sequential segmental analysis of the atria, ventricles, and great arteries and their connections will be performed. All fetal studies will be performed by Professor Fionnuala Breathnach and Dr Ann McHugh. Fetal echocardiography and hyperoxygenation administration will all be conducted on a GE Volusson E8 in a dedicated cubicle in the Fetal Assessment Unit, during a protected research session. All studies will be archived and reviewed later to assess quality and accuracy of data acquisition. Doppler measurement of the blood flow pattern in the first branch of either the right or left pulmonary artery (resistance and pulsatility indices) as well as peak systolic flow of pulmonary arterial blood velocity will be assessed before and after maternal inhalation for 10 minutes of 60% FiO2. The following measurements specific to the pulmonary artery Doppler waveform will be recorded:

1. The peak systolic velocity (Vsyst)
2. The time averaged maximum velocity (Vmean)
3. The time velocity integral (TVI; defined as the area under the velocity spectral envelope)
4. The pulsatility index (PI; defined as the difference between peak systolic and diastolic velocity divided by time averaged velocity)
5. The ejection time (ET; defined as the whole time of systole)
6. The acceleration time (AT; defined as the time from the initial increase in velocity to the time of peak velocity).

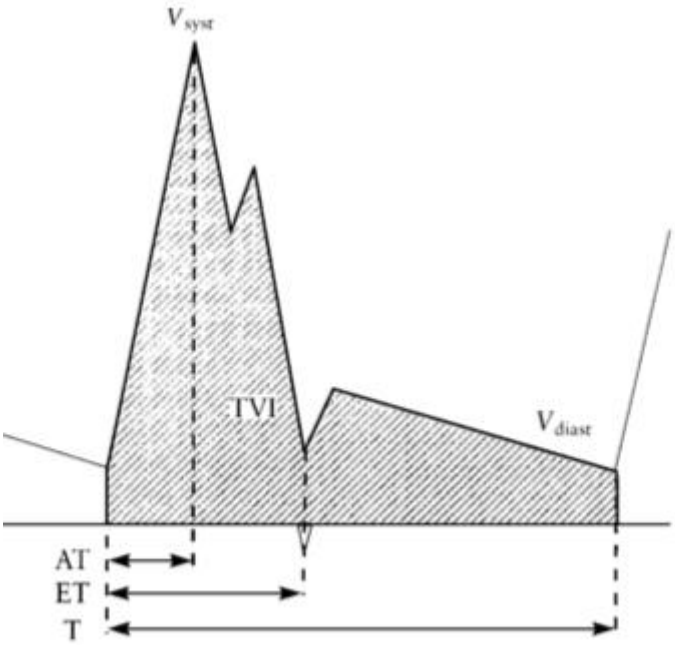


Figure 1:
Illustrates the Doppler flow velocity waveform in the pulmonary artery: a unique Doppler wave form pattern characterized by a rapid initial flow acceleration phase and an equally rapid deceleration phase, producing a needle-shaped systolic peak. This is followed by a more gradual decline in flow velocity, which is interrupted by a short reverse flow pattern at the beginning of diastole.

Oxygen which is well-used and safe in pregnancy will be administered to the patients while in a semi recumbent position in the hospital ultrasound department. Oxygen will be administered at a rate of 8-10L/min for a duration of 10 minutes via a partial non-rebreather mask. The ultrasound department in our hospital is equipped to administer oxygen to the patients, using a portable oxygen cylinder and disposable plastic non rebreather masks. Following maternal hyperoxygenation a repeat fetal echocardiogram will be performed. The hyper oxygenation test will be considered positive when the pulsatility index (PI) of the fetal pulmonary artery decreases by more than 20% of its baseline (responders). Where the fetal pulmonary arterial PI does not decrease by at least 20%, cases will be classified as non responders. Measurements of the Umbilical artery (UA) pulsatility index (PI), ductus venosus, ductus arteriosus PI, amniotic fluid indices and routine fetal biometry and estimated fetal weight measurements based on the Hadlock formula will be obtained as well as fetal heart rate variation.

In addition to a segmental analysis, the following connections will be evaluated: The Atrioventricular junction will be assessed for its anatomy, size, and function of atrioventricular (eg, mitral and tricuspid) valves; and the Ventriculoarterial junction for its anatomy, size, and function of semilunar (eg, aortic and pulmonary) valves, including assessment of both the subpulmonary and subaortic valves.

The rationale for why the MH test might be informative is because a high-resistance circulation in the pulmonary vascular bed exists during fetal life. Normal transition to newborn circulation

requires for high fetal pressures to fall, with dilatation of the pulmonary vessels. Previous studies have indicated that the capacity of these vessels to dilate can be judged by administering high-dose oxygen to the mother [5-7].Values for reactivity are based on previous studies which used a decrease in the PI of the pulmonary arteries by more than 20% of its baseline to characterize responders [5, 30].

We hypothesize that in fetuses who will develop persistent pulmonary hypertension as a neonate, the PI values in the pulmonary artery will be non reactive (a decrease of <20% in the PI) following maternal hyperoxygenation. This may help in identifying those fetuses with a poorer neonatal outcome.

Maternal haemodynamics will be monitored using the NICOM system over 30 minutes (ten minutes pre oxygen therapy, during the hyper oxygenation for 10 minutes and for 10 minutes following hyper oxygenation. Measurements of maternal cardiac output, stroke volume and systemic vascular resistance will be recorded. This is assessed non-invasively by placing 4 stickers on the mother's back for the duration of the study

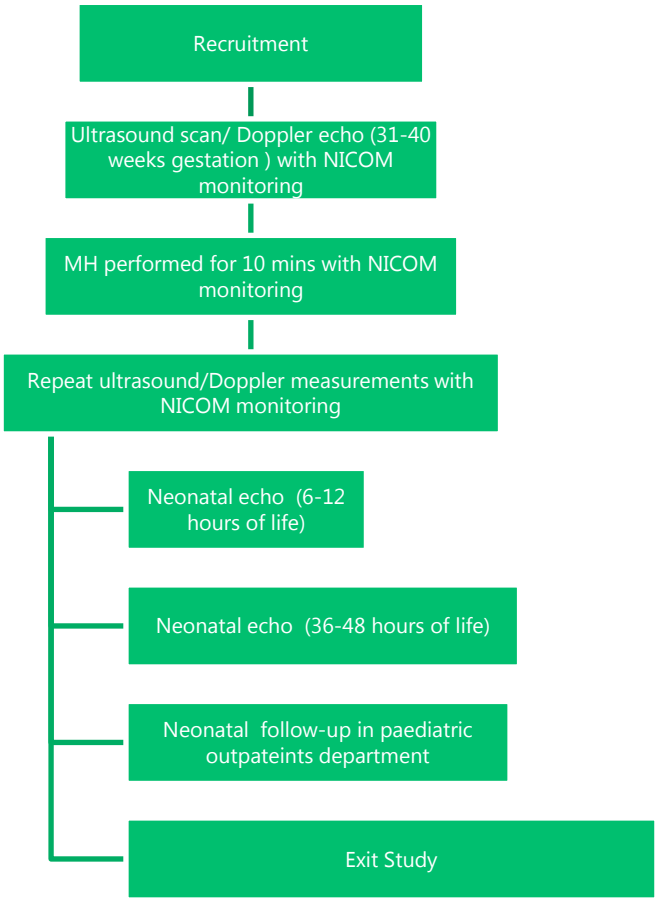
The presence of pulmonary hypertension in the neonate will be formally assessed with a neonatal echocardiogram performed at two separate time points, at 6-12 hours of life and at 36-48 hours of life. Persistent pulmonary hypertension will be defined by echocardiography as well as by clinical indicators as follows:
1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,
2) Normal Structural anatomy of the heart on echocardiogram; and,
3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or
4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic
5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity.
All neonates will be followed up in the paediatric outpatient department six weeks following discharge from hospital. All neonates will also undergo a cranial ultrasound prior to discharge from hospital.

12 TRIAL DESIGN

12.1 General considerations

This is a pilot study. All data will be collected prospectively. The study will evaluate the use of fetal echocardiographic Doppler assessment of the pulmonary vasculature prior to and following maternal hyperoxygenation therapy to predict fetuses at risk of pulmonary hypertension in the neonatal period. The Study will be undertaken in the Rotunda Hospital, Dublin.

Figure 1: Study Schema



- Test period – 30 minutes total
- NICOM monitoring period 30 minutes (10 minutes pre MH, 10 minutes during MH and 10 minutes after MH)
- Maternal test requires one visit at the ultrasound department with 2 ultrasound scans performed during that time.
- Neonatal test requires two neonatal echocardiograms within the first 48 hours of life
- Oxygen will be administered via a face mask, inhalation for 10 minutes of 60% FiO2.

12.2 Selection of study population

12.2.1 Overall description of trial subjects

Pregnant women who are carrying a fetus at risk of persistent pulmonary hypertension will be identified through the hospital records system (Current Inpatients, ultrasound department [anatomy scans], fetal medicine MDT meetings), and will be offered participation in the study as

part of a comprehensive fetal echocardiography. Pregnant women attending for scheduled caesarean sections prior to 38 weeks gestation will also be recruited to the study. Fifteen to thirty women with normal pregnancies will be recruited to participate in the study.

12.2.2 Inclusion criteria

The following subgroups of patients will be recruited to the study:

A) Women who are carrying a fetus at risk of pulmonary hypoplasia:
   a. Mid-trimester PPROM
   b. Persistent oligohydramnios
   c. Congenital diaphragmatic hernia/ CCAM
   d. Skeletal dysplasia

B) Women attending for scheduled Caesarean delivery prior to 38 weeks' gestational age

C) Women with a prenatal diagnosis of moderate/ severe perimembranous ventricular septal defect/ AVSD in the fetus in the absence of other structural heart disease including foetuses with Trisomy 21.

D) Fifteen to thirty patients with normal pregnancies will be recruited to the study that are not known to have a risk of PPHN. 15 to 30 patients will be recruited to allow for drop out of those patients who initially fall into the normal control group but may for whatever reason during the pregnancy fall into one of the other three groups as outlined above.

- Subjects must be able and willing to give written informed consent and to comply with the requirements of this study protocol
- Subjects must be female, aged 18 years or above at Baseline
- Subjects who are judged to be in generally good health by the investigator based upon the results of the medical history

12.2.3 Exclusion criteria

- Age < 18 years

- Known fetal chromosomal abnormality excluding Trisomy 21
- Gestational age <18 weeks and >40 weeks
- Maternal chronic respiratory disease (including COPD, Cystic Fibrosis, Pulmonary Fibrosis)
- Maternal congenital heart disease
- Maternal use of bleomycin or amiodarone
- Current maternal use of nitrofurantoin or use within the last 7 days.
- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder (including uncontrolled diabetes,
  unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
- Prior or concurrent malignancy

12.3 Study assessments and procedures

Figure 2: Schedule of events

Procedures	Visit 1 Screen/ Recruitment	Visit 2 31-40 weeks gestation	Visit 3 6-12hrs of neonate life	Visit 4 36-48 hours of neonate life	Visit 5 (6 weeks following discharge from hospital)
Inclusion/Exclusion Criteria	X	X			
Informed consent	*X				
Medical history	X	X			
Physical examination and weight/height	X	X			
Vital signs	X	X			
Concomitant medications	X	X			
Ultrasound scan and Doppler echo		X			
Hyperoxygenation for 10 minutes 60% FiO2		X			
Repeat ultrasound and Doppler echo		X			
NICOM		X			

assessment over the 30minutes of testing					
Neonatal weight/ demographics			X		
Neonatal echo (6-12hrs of life)			X		
Neonatal echo (36-48hours of life)				X	
Neonatal follow-up in paediatric outpatient department 6 weeks after hospital discharge					X

*Informed consent will be obtained prior to any study-related procedures being undertaken.

Study Procedure:

Doppler echocardiography will be performed on fetuses between 31-40 weeks gestation. A fetal echocardiogram will be performed according to an agreed protocol. This will involve a sequential segmental analysis of the atria, ventricles, and great arteries and their connections. Doppler measurement of the blood flow pattern in the first branch of either the right or left pulmonary artery (resistance and pulsatility indices) as well as peak systolic flow of pulmonary arterial blood velocity will be assessed before and after maternal inhalation for 10 minutes of 60% FiO2. The following measurements specific to the pulmonary artery Doppler waveform will be recorded:

1. The peak systolic velocity (Vsyst)
2. The time averaged maximum velocity (Vmean)
3. The time velocity integral (TVI; defined as the area under the velocity spectral envelope)
4. The pulsatility index (PI; defined as the difference between peak systolic and diastolic velocity divided by time averaged velocity)
5. The ejection time (ET; defined as the whole time of systole)
6. The acceleration time (AT; defined as the time from the initial increase in velocity to the time of peak velocity).

Oxygen will be administered to the patients while in a semi recumbent position in the hospital ultrasound department. Oxygen will be administered at a rate of 8-10L/min for a duration of 10 minutes via a non-rebreather mask. The ultrasound department in our hospital is equipped to administer oxygen to the patients, using a portable oxygen cylinder and disposable plastic non rebreather masks. Following maternal hyperoxygenation a repeat fetal echocardiogram will be

performed. The hyper oxygenation test will be considered positive when the pulsatility index (PI) of the fetal pulmonary artery decreases by more than 20% of its baseline (responders). Where the fetal pulmonary arterial PI does not decrease by at least 20%, cases will be classified as non responders. Measurements of the Umbilical artery (UA) pulsatility index (PI), Amniotic fluid indices and routine fetal biometry and estimated fetal weight measurements based on the Hadlock formula will be obtained as well as fetal heart rate variation.

In addition to a segmental analysis, the following connections will be evaluated: The Atrioventricular junction will be assessed for its anatomy, size, and function of atrioventricular (eg, mitral and tricuspid) valves; and the Ventriculoarterial junction for its anatomy, size, and function of semilunar (eg, aortic and pulmonary) valves, including assessment of both the subpulmonary and subaortic valves.

We hypothesise that in fetuses who will develop pulmonary hypertension as a neonate values in the pulmonary artery will be non reactive (a decrease of <20% in the PI) following maternal hyperoxygenation. This may help in identifying those fetuses with a poorer neonatal outcome. Maternal haemodynamics will be monitored using the NICOM system over 30 minutes (ten minutes pre oxygen therapy, during the hyper oxygenation for 10 minutes and for 10 minutes following hyper oxygenation. Measurements of maternal cardiac output, stroke volume and systemic vascular resistance will be recorded.

The presence of pulmonary hypertension in the neonate will be formally assessed with a neonatal echocardiogram performed at two separate time points, at 6-12hours of life and at 36-48 hours of life. Persistent pulmonary hypertension will be defined by echocardiography as well as by clinical indicators as follows:

1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,
2) Normal Structural anatomy of the heart on echocardiogram; and,
3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or
4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic
5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity.

Fetal Echocardiogram Examination Protocol (Full explanation of Protocol attached)

General measurements:

1. Fetal biometry: HC, BPD,AC, FL
2. Amniotic fluid index
3. PW Doppler of the Umbilical Artery (include PI)

Fetal Echo:

1. Image quality
2. Situs
3. Position of stomach
4. Heart size
5. Position of apex
6. Systemic veins
7. Pulmonary veins
8. Atrial septum
9. Flow at foramen ovale
10. Atrioventricular Junction
11. Atrioventricular valve regurgitation
12. Ventricular septum
13. Ventricular function
14. Great artery connections
15. Arterial valve regurgitation
16. Branch pulmonary artery
17. Ductus arteriosus
18. Aortic arch
19. Side of arch
20. Subclavian arteries
21. Rhythm
22. Rate
23. Pulmonary arteries (averaged from the values obtained from three cardiac cycles)
    - 2D measurements
    - Color
    - PW Doppler (proximal- just after takeoff)
    - Pulsatility index
    - Resistance Index
    - Vsyst
    - Peak systolic velocity
    - Vdiast
    - enddiastolic velocity
    - TVI- time velocity integral
    - AT-acceleration time
    - ET-ejection time

Post hyperoxygenation
10minutes of 60% FiO2 via non re-breather mask

Measurements:

1. PW Umbilical artery Doppler
2. Fetal heart rate
3. Pulmonary arteries (averaged from the values obtained from three cardiac cycles)
    - 2D measurements
    - Color
    - PW Doppler (proximal- just after takeoff)
    - Include PI
    - Vsyst
    - peak systolic velocity
    - Vdiast
    - enddiastolic velocity
    - TVI- time velocity integral
    - AT-acceleration time
    - ET-ejection time

12.3.1 Description of Study Assessments

Medical and Surgical History

Any medical history in the mother will be recorded. Particular attention will be payed to any maternal chronic respiratory disease (including COPD, Cystic Fibrosis, Pulmonary Fibrosis), maternal congenital heart disease, maternal use of bleomycin or amiodarone or those with any other significant disease or disorder (including uncontrolled diabetes,
unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident)

Demographics

The following maternal details will be collected at enrolment:
Maternal age
Parity and Obstetric history
Weight and height (BMI calculation)
Socioeconomic grouping
Ethnicity
Smoking, alcohol and drug use
Medical history
Medication use
Antenatal administration of corticosteroids
Antenatal use of tocolytics
Gestational age at PPROM (weeks) if relevant
Interval from PPROM- delivery (days) if relevant

The following perinatal data will be collected:
Time interval between oxygen test and delivery
Fetal sex
Birth weight
Gestational age at delivery
Gestational age at maternal hyperoxygenation test
Indication for delivery

Physical Examination

The complete physical examination will include the evaluation of the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, neurological systems. Height, weight and blood pressure will also be recorded.

Vital Signs

Vital signs will be recorded for all subjects and will include: blood pressure (BP), temperature (°C), pulse, and respiratory rate. Vital signs will be obtained at Baseline, at each study visit and at the end of study. Resting pulse and blood pressure (BP) measurements will be measured after the subject has sat for at least five minutes.

ECG Test
N/A

Clinical Laboratory Tests

N/A

Pregnancy Tests

N/A

Concomitant Medication

All over-the-counter or prescription medication, vitamins, and/or herbal supplements will be recorded on CRFs. The indication for treatments will be recorded.

12.3.2 Endpoints assessments

Efficacy Assessment

The hyper oxygenation test will be considered positive when the pulsatility index (PI) of the fetal pulmonary artery decreases by more than 20% of its baseline (responders). Where the fetal pulmonary arterial PI does not decrease by at least 20%, cases will be classified as non responders.

Maternal haemodynamics will be monitored using the NICOM system over 30 minutes (ten minutes pre oxygen therapy, during the hyper oxygenation for 10 minutes and for 10 minutes following hyper oxygenation. Measurements of maternal cardiac output, stroke volume and systemic vascular resistance will be recorded.

Persistent pulmonary hypertension will be defined by echocardiography as well as by clinical indicators as follows:
1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,
2) Normal Structural anatomy of the heart on echocardiogram; and,
3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or
4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic
5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity.

Safety Assessment

The following safety evaluations will be performed during the study: adverse event monitoring, vital signs, physical examination.

12.3.3 Screening procedure

Participants will be selected from the hospital fetal medicine ultrasound scan lists, inpatient records and operating theatre lists. Patients attending the fetal medicine ultrasound scan lists with foetuses at risk of pulmonary hypoplasia will be given an information leaflet and consent form. As they will be seen regularly at the scan lists they will be recruited at their next visit if over 31 weeks gestation. Patients booked for elective caesarean sections prior to 38 weeks gestation, will be identified on theatre lists and contacted by telephone by Dr McHugh. They will be asked to partake in the study and an information leaflet and consent form will be posted to their address. During the screening period, subjects will be evaluated for eligibility. Date of screening, subject age, and reason for ineligibility will be recorded. The results of the screening evaluation must meet the inclusion/exclusion criteria for the subject to continue in the study.
If agreeable they will be asked to attend for an ultrasound scan and fetal echocardiogram prior to their scheduled caesarean section. At that time they will sign the consent form. Informed consent will be obtained prior to any study related procedures being undertaken.

12.3.4 Baseline assessments

N/A

12.3.5 Subsequent study visits and procedures

Visit 1 (in person)

- Informed consent obtained
- Eligibility check
- Assessment of efficacy outcome measures
- Assessments of safety (adverse event monitoring, vital signs, physical examination)

Visit 2 (in person in ultrasound department)

- Ultrasound scan including fetal echocardiogram
- NICOM (x4 stickers) monitoring for 10 minutes prior to MH
- Hyperoxygenation for 10 minutes while lying semirecumbent, administered by face mask. 60% FiO2
- NICOM monitoring during MH
- Repeat ultrasound/ echocardiogram with NICOM monitoring

Visit 3

- Neonatal echocardiogram at 6-12 hours of life.

Visit 4

- Neonatal echocardiogram at 36-48 hours of life.

Visit 5
Neonatal follow-up in paediatric outpatient department 6 weeks following discharge from hospital.

12.3.6 Method of assigning Subjects to treatment groups

Randomisation

N/A

Blinding

N/A

12.4 Definition of end-of-trial

The end of trial will be the date of the last visit of the last subject.

The end of the study will be reported to the REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. In the case of premature termination the investigators will inform subjects and ensure that the appropriate follow-up is arranged for all involved.

A summary report of the study will be provided to the REC and Regulatory Authority within 1 year of the end of the study and within 6 months for paediatric studies as this is a legal requirement.

12.4.1 Premature termination of the study

The study will be prematurely terminated if new information about the safety or efficacy, of hyperoxygenation emerges or if there is unsatisfactory progress of the study.

12.5 Discontinuation/withdrawal of subjects from study protocol

Subjects have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason without any consequences. The investigator has the right to discontinue a subject from study treatment or withdraw a subject from the study at any time if it is in the best interest of the subject.
Subjects must discontinue the investigational medicinal product(s) and be withdrawn from the study for any of the following reasons:

- Withdrawal of consent by the subject
- Any medical condition that the investigator or sponsor determines may jeopardize the subject's safety if she or he continues receiving the study treatment.

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- An adverse event which requires discontinuation of the study
- Lack of compliance with the study and/or study procedures (e.g., follow up neonatal visits)
- Lost to follow-up. At least 3 documented attempts will be made to contact any subject lost to follow-up.

All subjects who discontinue should comply with protocol specified follow-up procedures. The only exception to this requirement is when a subject withdraws consent for all study procedures.

If a subject is withdrawn before completing the study, the reason for withdrawal must be entered on the appropriate case report form (CRF) page.

If a subject is withdrawn due to an adverse event, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilised.

13 TREATMENT OF TRIAL SUBJECTS

13.1 Description of study treatment(s)

The trade name of the medicinal product is medical oxygen. The name of the active substance is oxygen. The formulation is 100% V/V inhalational gas. The marketing authorisation number in Ireland is PA1357/001/001. The marketing authorisation holder is Industrial Pressure testing Ltd

The dose will be 60% FiO2 administered via a face mask over ten minutes.
This dosing has been used in numerous studies previously without any adverse effects.

13.2 Formulation, packaging and handling

Product name: Medical Oxygen 100% v/v Inhalational Gas
ATC code V03 AN01
Pharmaceutical form 100% v/v Inhalational Gas
Maximum duration of treatment of a subject according to the protocol: 10 minutes
Dose allowed: 8-10Litres/minute
Route of administration Inhalation through the lungs
Name of each active substance: Oxygen
Marketing Authorisation Holder and Manufacturer BOC Gases Ireland Limited. J F Kennedy Drive Bluebell Dublin 12 Tel 1800 370700 healthcareinfo.ie@boc.com

13.3 Storage and disposition of study treatment(s)

Oxygen will be stored in appropriate oxygen cylinders

13.4 Accountability of the study treatment(s)

The study medication will be supplied to pharmacy by BOC Gases Ireland Limited. The investigator is responsible for the control of the treatment under investigation. Adequate records for the receipt and disposition of the IMP must be maintained. The investigator will use a standard prescription form and the investigator/research nurse will collect the medication from the pharmacy. The oxygen cylinder will be stored in the ultrasound department.

13.5 Assessment of compliance

The investigator is responsible for ensuring that the study treatment is administered in compliance with the protocol. Subject compliance will be assessed by witnessing the oxygen administration in the room.

13.6 Overdose of study treatment

N/A

13.7 Prior and concomitant therapy

Any medication, other than the study medication taken during the study will be recorded in the CRF.

The timeline for documenting medications is at the recruitment visit. Prior and concomitant medication taken by the subject up to and including 7 days prior to the recruitment visit will be documented on the CRF.

13.7.1 Permitted medications/non- investigational medicinal products

Any medication that the patient has been prescribed or is taking themselves is permitted. This excludes the use of bleomycin or amiodarone.

13.7.2 Prohibited medications

Bleomycin and amiodarone. Current maternal use of nitrofurantoin or use within the last 7 days.

14 SAFETY REPORTING

The safety of the investigational medicinal product (IMP) will be assessed through the recording, reporting and analysing of baseline medical conditions, adverse events, vital signs, physical exam findings in line with GCP. The study investigators will adhere to all protocols, safety procedures and Standard Operating Procedures (SOPs) as set out by the sponsor (RCSI). All adverse events will be collected and recorded in the CRF. A line listing of adverse events will be reported to the national competent authority in Ireland, the HPRA on an annual basis.

14.1 Definitions

14.1.1 Adverse event (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product

14.1.2 Adverse reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose.
The phrase 'responses to a medicinal product' means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.
All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

14.1.3 Serious adverse event

Any untoward medical occurrence or affect that at any dose:

- results in death,
- is life-threatening*,
- requires hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect
- important medical events**

*Regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

**Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious' in accordance with the definition

Important adverse events that may not result in death, may not be life-threatening, or do not require hospitalization may be considered Serious Adverse Events (SAE) when, based upon appropriate medical judgment, they may jeopardize the research participant or may require medical or surgical intervention to prevent one of the outcomes listed above. Hospitalisation for elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and did not worsen will not be considered to be an SAE.
Abnormal laboratory findings and other objective measurements will be captured routinely and NOT reported as AEs as they will be collected and analyzed separately. However, abnormal laboratory findings or other objective measurements that meet the criteria for a SAE, result in discontinuation of the IMP, require medical intervention or are judged by the investigator to be clinically significant changes from baseline values will be captured and reported on the AE pages of the CRF.

14.1.4 Severe adverse events

The term 'severity' is used here to describe the intensity of a specific event. This has to be distinguished from the term 'serious.

14.1.5 Suspected unexpected serious adverse reactions

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational medicinal product or summary of product characteristics for an authorised medicinal product.

14.2 Evaluation of AEs and SAEs

Seriousness, causality, severity and expectedness should be evaluated.

14.2.1 Assessment of seriousness

The Principal Investigator (PI) should make an assessment of seriousness as defined in section 14.1.3.

14.2.2 Assessment of casualty

All adverse events judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions.
The causality assessment given by the investigator should not be downgraded by the sponsor.
The investigator/sponsor must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions:

The following are regulatory definitions

Unrelated

Where an event is not considered to be related to the study medication.

Unlikely

Where an event is considered unlikely to be related to the study medication

Possibly

Although a relationship to the study medication cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably

The temporal relationship and absence of a more likely explanation suggest the event could be related to the study medication.

Definitely

Where an event is considered to be definitely related to the study medication
All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably) to the study medication will be considered as ARs/SARs.

All AEs/SAEs judged as being related (e.g. definitely, possibly, probably) to an interaction between the study medication and another medication will also be considered to be ARs/SAR.

Alternative causes such as natural history of the underlying disease, concomitant therapy, other risk factors and the temporal relationship of the event to the treatment should be considered.

14.2.3 Assessment of severity

The investigator will make an assessment of severity for each AE/SAE and record this on the CRF. The severity of AEs will be graded using the most current version of the Common Terminology Criteria for Adverse Events (CTCAE) 5-point scale:

- **Mild (Grade I)**: Asymptomatic or mild symptoms: clinical or diagnostic observations only; intervention not indicated.
- **Moderate (Grade II)**: Minimal, local or non-invasive intervention indicated; limited age-appropriate instrumental activities of daily living (ADL).
- **Severe (Grade III)**: Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- **Life-threatening (Grade IV)**: Life-threatening consequences; urgent intervention indicated.
- **Death (Grade V)**: Death related to AE.

If an AE has multiple aspects, the aspect with the highest severity will be graded. Note: the term 'severe', should not be confused with 'serious' which is a regulatory definition based on subject/event outcome or action criteria>

14.2.4 Assessment of expectedness

The expectedness of an adverse reaction will be determined by the sponsor according to the reference document for the study medication; the summary of product characteristics (SPC) for medical oxygen. SAEs that are determined to be unrelated to the study medication do not need an expectedness assessment against the reference safety document to assess whether the event meets SUSAR criteria.

14.2.5 Emergency unblinding procedures

N/A

14.3 Reporting procedures for all adverse events

All AEs occurring during the study observed by the investigator or reported by the subject, whether or not attributed to the study medication, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to the study medication, other suspect medication or device and action taken and outcome. Follow-up information should be provided as necessary.

AEs considered related to the study medication as judged by an investigator or the sponsor will be followed until resolution or until the event is considered stable. All related AEs that result in a subject's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the subject's removal from treatment. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the subject must undergo an end-of-study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The severity of events will be assessed on the scale outlined in Section 14.2.3

The relationship of AEs to the study medication will be assessed by the investigator as outlined in Section 14.2.2.

14.4 Reporting procedures for serious adverse events

The Principal Investigator (PI) or appropriate designee is responsible for capturing all AEs and SAEs on appropriate trial specific AE and SAE forms, reporting all SAEs to RCSI Pharmacovigilance within 24 hours of first becoming aware of the event (as per the RCSI Sponsor SOP on Expedited Safety Reporting). It is important to note that in the event that an SAE was not previously documented as an AE, the PI or designee must also fill out an AE form in junction with an SAE form.
SAEs will be collected from the time of the subject's consent until the final study visit. SAEs that are related to the investigational drug and continue beyond the normal collection period (i.e., are ongoing at the time a subject exits the study) will be followed until resolution or until stabilized with sequelae. SAEs that begin after the subject's participation in the study is complete, but that the PI considers to be related to study drug, may be reported at any time.
The process for reporting SAEs is as follows:

- The investigator or designee will report all serious adverse events on a trial specific paper SAE form and scan and email to: pharmacovigilance@rcsi.ie within 24 hours of becoming aware of the event along with supporting source documents (e.g. laboratory reports),

The initial SAE report must contain at least;

- o An identifiable clinical trial participant: identified using a clinical trial specific code
- o Reference to the study medication under investigation
- o A description of the SAE (ideally the diagnosis otherwise the signs and symptoms until the diagnosis is confirmed)
- o Reporter ID: The report must contain information that clearly identifies the reporter (name, position, contact details)

- The PI will assign the severity, causality and serious criteria of the event as per Section 9.2 of the protocol, and will document it on the SAE form.

- The sponsor may contact the PI or site designee to request clarification or additional information (such as applicable source data) if required. In the event of follow up information being required; the PI or site designee should then return follow up information to: pharmacovigilance@rcsi.ie by returning a follow-up SAE form. The initial and follow-up reports will identify subjects by unique code numbers assigned to the latter.

- The assessment of expectedness is done by the Sponsor using the RSI located within the IB or SmPC. Note: based on the PI's assessment of causality; the sponsor may upgrade the

causality but may not downgrade it. If information on expectedness has been made available by the reporting PI than this should be taken into consideration.

- The sponsor will forward the SAE report along with the SAE Chief Investigator Internal Review Form and clinical trial specific SAE query form to the CI for medical review by email in a timely manner. A telephone call may also be made to avoid any delays. For clarification/confirmation purposes the telephone call will be followed up with an email.

- The CI will medically review the SAE and follow-ups within 1 working day and return any comments to pharmacovigilance@rcsi.ie on the CI Internal Review Form. (In some cases, the sponsor may request the CI to assign medical opinion of causality and expectedness using the RSI located within SmPC). Note: based on the PI's assessment of causality; the CI may upgrade the causality but may not downgrade it.

- If an SAE is assigned as related to the IMP, it is designated as a SAR. If an SAR is assessed as unexpected, by either the reporting PI or the sponsor, it is designated as a SUSAR.

- The sponsor will report all SUSARs to the competent authorities (the HPRA in Ireland) and the ethics committees concerned. Fatal or life-threatening SUSARs must be reported to Eudravigilance within 7 calendar days. SUSARs which are not fatal and not life-threatening are to be reported to Eudravigilance within 15 calendar days. As the HPRA will report SUSARs to Eudravigilance on behalf of the sponsor, the sponsor must report SUSARs to the HPRA within shorter timelines as defined by the HPRA before the start of the study. The sponsor will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects. This will be reported to Investigators in a blinded fashion.

If the initial report is incomplete, e.g. if the sponsor has not provided all the information/assessment within seven days, the sponsor will submit a completed report based on the initial information within an additional eight days.
If significant new information on an already reported case is received by the sponsor, the clock starts again at day zero, i.e. the date of receipt of new information. This information will be reported as a follow-up report within 15 calendar days of the initial reporting of a fatal/life threatening SUSAR.

In addition to the expedited reporting above, the sponsor shall submit once a year throughout the clinical trial or on request, a safety report to the competent authority (the HPRA in Ireland) and the relevant ethics committees. The annual safety report will be presented in the DSUR format as per ICH guideline E2F - Note for guidance on development safety update reports. This is a legal requirement.
Medical and scientific judgement will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the research participant or may require intervention to prevent one of the other outcomes listed in the definition above. These will be considered also serious.

14.5 Data safety monitoring board (DSMB)

To provide protection for study participants, an independent DSMB has been appointed for the planned trial to oversee the safety monitoring. The DSMC members will perform an objective

oversight function, based on their lack of conflict of interest or involvement within the trial. The DSMC will meet at time points outlined in the DSMB Terms of Reference document and will provide reports to the Trial Management Group regarding the continuing safety of current participants and those yet to be recruited, as well as reviewing the validity and scientific merit of the trial. The DSMB composition, name, title and address of the chairman and of each member, will be given in the DSMB Terms of Reference. The independent statistician will provide the analysis service required by the DSMB. This Terms of Reference includes, but is not limited to, defining the following:

- Schedule and format of the DSMB meetings
- Format for presentation of data
- Method and timing of providing interim reports
- Stopping rules

To enable it to respond to any safety signal, the DSMB will be fully functional before enrolment into the trial begins. Operating procedures describing how the DSMB works and how it communicates with other study participants (e.g. with the Data Manager or the Sponsor) will be in place before enrolment into the trial begins.
The members of the DSMB will be outlined in the DSMB terms of reference document.

14.6 Trial Management Group (TMG)

The study will be overseen by Dr Fionnuala Breathnach. The investigators will meet quarterly. A data safety and monitoring board will assess the quality and timeliness of data collection, review the safety data and report to the investigators (see Section 14.5). Dr Fionnuala Breathnach will serve as the project principal investigator (PI), thereby assuming overall responsibility and control for the project and will coordinate the contributions of other partners involved. The financial and administrative aspects of the study will be administrated with the assistance of the Friends of the Rotunda. The Programme Manager (PM) will co-ordinate the day-to-day management of the project and will liaise with the team on actions to be undertaken to ensure the project proceeds in a timely manner. The PI, PM and statistician will meet with the RCSI Quality and Regulatory Affairs Manager (QRAM) at least once per month. The PM will organise and run these meetings, define agendas, participate as chairperson and circulate minutes. Regular site visits and teleconferences will also take place.

Additional Research Staff
A research assistant will be appointed and charged with acquisition of blood samples and data input. A Lead Researcher (LR) will be appointed to drive forward this project with a view to completing a PhD thesis on this work, under the direct supervision of Dr Breathnach. Provision of a salary for the LR will not be required HRA - POR 2014 HRA v1.0 Reference: HRA-POR-2014-635 Date submitted: 30/10/2013 Page 11 of 66 through this grant as he/she will occupy an existing salaried lecturer post. The research team has access to a biostatistician through Perinatal Ireland, providing expertise on all aspects of biostatistical design, analysis and data management, in addition to preparation of publications. Finally, clinical research staff for phlebotomy are also available.

Performance Monitoring
The PM and LR will present regular reports to the research team. These will include:
• Monthly recruitment reports - a report of the number of women enrolled by month.

• Quarterly research reports - a report detailing recruitment, baseline patient characteristics, data quality, incidence of missing data and adherence to study protocol.

Data Management

Data will be managed through entry into an encrypted, password protected database with double entering of every 10th data point to assess for data entry accuracy. The centralized database will be located at the Royal College of Surgeons Ireland for statistical analysis and patients will be allocated study numbers. Only the LR, the statistician, PM and PI will have access. A data safety monitoring board will be established to oversee any safety issues that may arise. At regular intervals, audits, which compare data across forms, will be run by the Biostatistician on the entire database or on a specific subset of data. An audit trail will be maintained so that the succession of corrections can be monitored.

In order to plan and manage the communication of outputs and achievements from the study, a clear dissemination and knowledge exchange is in development. This includes dissemination of both the scientific and medical outputs and of the clinical impact that may potentially change patient management. Target audiences include specialist healthcare professionals, the general medical and scientific community, study participants, patient groups where appropriate and the wider community.

Publications, reports and presentations:

All the partners involved in the project will contribute throughout Europe and North America to disseminate information about the project and its results at conferences and seminars e.g. Society of Maternal Fetal Medicine Meeting, British Maternal Fetal Medicine Society Meeting amongst others. It is forecasted to publish at least two to three research articles in specialised peer-review journals throughout the lifetime of this project. The research findings will be communicated at national and international meetings as lectures and posters.

The Following Work package has been developed for the Study.

WP 1.1: Patient recruitment:

The principal investigator is involved in Perinatal Ireland and has a vast experience in large scale trials and patient recruitment. Recruitment for this study will be done in the Rotunda Hospital. We aim to recruit 60- 75 patients in total. Patients attending the fetal medicine ultrasound scan lists with a fetus at risk of persistent pulmonary hypertension will be given an information leaflet and consent form. As they will be seen regularly at the scan lists they will be recruited at their next visit if over 31 weeks' gestation. Patients booked for elective caesarean sections prior to 38 weeks gestation, will be identified on theatre lists and contacted by telephone by Dr McHugh. They will be asked to partake in the study and an information leaflet and consent form will be posted to their address. If agreeable they will be asked to attend for an ultrasound scan and fetal echocardiogram prior to their scheduled caesarean section. At that time they will sign the consent form.

WP 1.2: Measurement of the Hemodynamic profile

Hemodynamic monitoring will be performed with the patient lying semi-recumbent, slightly tilted to the left to avoid aorto-caval compression. Four double NICOM® electrodes will be attached, 2 below the clavicle in the mid clavicular line and 2 at the costal margin in the mid-clavicular line. A non-invasive blood pressure cuff will also be placed on the

patients' upper arm to measure brachial artery pressure at 5 minute intervals. Hemodynamic monitoring will be started at this point and continued for 30 minutes, (ten minutes pre oxygen therapy, during the hyper oxygenation for 10 minutes and for 10 minutes following hyper oxygenation) Measurements of maternal cardiac output, stroke volume and systemic vascular resistance will be recorded.

Tasks

- Identification of potential participants, patient recruitment, maternal NICOM
- Monitoring of research activities - adherence to study protocol, monitoring of recruitment targets, data quality
- Analysis of results & population of research database

Deliverables

1.1 Kick-off Meeting held & Initiation of patient recruitment
1.2 Monthly recruitment charts
1.3 Data analysis and interpretation

Milestones

- Recruitment targets achieved and recruitment phase completed
- Completion of all maternal hemodynamic assessments

WP 1.3 Performing Fetal Echo and the Hyperoxygenation test:

All fetal studies will be performed by Professor Fionnuala Breathnach and Dr Ann McHugh. Fetal echocardiography and hyperoxygenation administration will all be conducted on a GE Volusson E8 in a dedicated cubicle in the Fetal Assessment Unit, during a protected research session. All studies will be archived and reviewed later to assess quality and accuracy of data acquisition. Doppler echocardiography will be performed on fetuses between 31-40 weeks gestation. A fetal echocardiogram will be performed according to an agreed protocol. This will involve a sequential segmental analysis of the atria, ventricles, and great arteries and their connections. Doppler measurement of the blood flow pattern in the first branch as well as peak systolic flow of pulmonary arterial blood velocity will be assessed before and after maternal inhalation for 10 minutes of 60% FiO2.

Oxygen will be administered to the patients while in a semi recumbent position in the hospital ultrasound department. Oxygen will be administered at a rate of 8-10L/min for duration of 10 minutes via a non-rebreather mask. The ultrasound department in our hospital is equipped to administer oxygen to the patients, using a portable oxygen cylinder and disposable plastic non rebreather masks. Following maternal hyperoxygenation a repeat fetal echocardiogram will be performed. The hyper oxygenation test will be considered positive when the pulsatility index (PI) of the fetal pulmonary artery decreases by more than 20% of its baseline (responders). Where the fetal pulmonary arterial PI does not decrease by at least 20%, cases will be classified as non-responders. Measurements of the Umbilical artery (UA) pulsatility index (PI), ductus venosus, Amniotic fluid indices and routine fetal biometry and estimated fetal weight measurements based on the Hadlock formula will be obtained as well as fetal heart rate variation.

In addition to a segmental analysis, the following connections will be evaluated: The Atrioventricular junction will be assessed for its anatomy, size, and function of atrioventricular (eg, mitral and tricuspid) valves; and the Ventriculoarterial junction for its anatomy, size, and function of semilunar (eg, aortic and pulmonary) valves, including assessment of both the subpulmonary and subaortic valves.

The following measurements specific to the pulmonary artery Doppler waveform will be recorded:
1. The peak systolic velocity (Vsyst)
2. The time averaged maximum velocity (Vmean)
3. The time velocity integral (TVI; defined as the area under the velocity spectral envelope)
4. The pulsatility index (PI; defined as the difference between peak systolic and diastolic velocity divided by time averaged velocity)
5. The ejection time (ET; defined as the whole time of systole)
6. The acceleration time (AT; defined as the time from the initial increase in velocity to the time of peak velocity).

Tasks

- Validate Echo assessments in study setting
- Perform fetal echocardiography assessment on all women recruited to study
- Analysis of results & population of research database

Deliverables

1.31 Monthly recruitment charts
1.32 Study reports generated
1.33 Data analysis and interpretation

Milestones

- Completion of all echocardiograms assessments and hyperoxygenation tests
- Data interpretation complete

WP 2.1: Neonatal echocardiography assessment

The echocardiography scans will be performed at two time periods: the first 6 to 12 hours and 36 to 48 hours after birth. Evaluations will be performed using the Vivid echocardiography system (GE Medical, Milwaukee) and a cardiology multi-frequency probe. The echocardiography machine and the necessary probes are already available at the Rotunda Hospital. The echo machine is primarily dedicated to research and will therefore be available for the study. All studies on asymptomatic infants will be performed in a dedicated quiet room in the postnatal floor when the infant is in a quiet state ideally after feeds. No sedation will be used. If the infant is admitted to the neonatal intensive care unit, the echocardiography study will be performed there. All scans will be recorded on the machine's internal hard drive and transferred to the EchoPAC archiving system for offline measurements and validation. The archiving system is also available at the Rotunda Hospital and has enough storage capacity for 15 years with an activity of 300 scans per year. Analysis of the parameters will be done while assessor is blinded to the hemodynamic profile and result of the hyperoxygenation test of the mother. The scans will all be performed by Dr Afif EL-Khuffash or Dr Colm Breathnach. Dr EL-Khuffash has over 10 years' experience in echocardiography of the term and preterm infants. The lead researcher Dr McHugh will keep track of all the study participants' delivery progress and alert the

echocardiographer of the infant's delivery. All studies will be archived and reviewed later to assess quality and accuracy of data acquisition. The first echocardiogram of each infant will undergo a formal examination to exclude any structural heart defects. Studies will be performed using standard neonatal windows including apical, parasternal, subcostal, and high parasternal windows. Persistent pulmonary hypertension will be defined by echocardiography as well as by clinical indicators as follows:
1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,
2) Normal Structural anatomy of the heart on echocardiogram; and,
3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or
4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic
5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity.

Tasks

- Perform echocardiography assessment on the infants of all women recruited to study
- Analysis of results & population of research database

Deliverables

2.1 Monthly recruitment charts
2.2 Study reports generated
2.3 Data analysis and interpretation

Milestones

- Completion of all neonatal cardiac assessments
- Data interpretation complete

WP 3: Dissemination and Knowledge Exchange Plan (Months 6-24)

The communication of outputs and achievements from this study will be disseminated to the following target audiences - specialist healthcare professionals, the general medical and scientific community, study participants, patient groups and the wider community. All partners will disseminate information about the project and its results at international conferences and seminars. It is planned to publish a number of research articles in specialised peer-review journals throughout the lifetime of this project. Book chapters and reviews will also be generated based on the expertise developed in the project. Outreach seminars aimed at the general public (women of child-bearing age) will be convened.

Tasks:

- Develop a communication / dissemination plan targeting major themes for publication in leading journals and for presentation at conferences
- Develop external communication channels to publicise outputs

Deliverables

3.1 Complete research database

3.2 Dissemination Plan
3.3 Present results/outcomes at Medical Conferences / Patient Group Annual Meetings
Milestones
Abstract and manuscript preparation

15 Statistics

15.1 Description of statistical methods

Descriptive statistics will be used to summarize the findings into two groups responders and non-responders.

The primary analysis of the primary outcome will be performed using an independent t-test (or a Wilcoxon Rank Sum test as appropriate) to compare the difference in PPHN severity in infants with and without a normal MH test. A chi squared test will be used for the primary analysis of the dichotomous secondary outcomes. For the continuous secondary outcomes, a t-test will be used to compare normally distributed data, and Wilcoxon Rank Sum test will be used for skewed data. We will accept a p value of < 0.05 as significant. We will use SPSS (version 22) to perform the statistical analysis.

15.2 Determination of sample size subjects

Sample sizes of between 24 (12 per group) and 50 have been recommended variously for pilot studies [refs 1,2,3, 4, below]. Following these broad recommendations and that of the NHS [ref 5], we chose a recruitment sample size of 60- 75 (15 per group plus a potential addition of 15 subjects in group D) which would allow for a moderate dropout rate. A significant dropout rate (e.g. 40%) would reduce the pilot sample size to below a minimum 24, in which case a planned larger study would be called into question in the first place, having possible external validity issues, pragmatic or ethical concerns.

[1]
Sim J, Lewis M.
The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency.
J Clin Epidemiol
2012;65:301-308

[2]
Julious SA.
Sample size of 12 per group rule of thumb for a pilot study.
Phrm Stat 2005;4:287-291

[3]
Lancaster GA, Dodd S, Williamson PR.
Design and analysis of pilot studies: recommendations for good practice.
J Eval Clin Practice. 2004;10:307-312

[4]
Recommendations for planning pilot studies in clinical and translational research.
Moore CG1, Carter RE, Nietert PJ, Stewart PW. Clin Transl Sci. 2011 Oct;4(5):332-7

[5] Justifying sample size for a feasibility study. National Institute for Health Research http://www.rdslondon.co.uk/RDSLondon/media/RDSContent/copy/Justifying-Sample-Size-for-a-Feasibility-Study.pdf This is a pilot study. The number of subjects will be 60 (n=60).This will be divided at 20 subjects per group category.

Most fetuses involved in the study are at risk of persistent pulmonary hypertension of the newborn. Fifteen to thirty subjects will be in the normal pregnancy control group. These fetuses are not known to be at increased risk of PPHN.
Whether from group A, B, C or D, we hypothesize that some subjects will have a reactive hyperoxygenation test and others will have a non-reactive hyperoxygenation test.

Therefore there will be 2 groups (responders and non-responders). We will use descriptive statistics to summarize the findings in both groups.

15.3 Analysis sets

Pregnant women who are carrying a fetus at risk of persistent pulmonary hypertension will be identified through the hospital records system (Current Inpatients, ultrasound department [anatomy scans], fetal medicine MDT meetings), and will be offered participation in the study as part of a comprehensive fetal echocardiography. Pregnant women attending for scheduled caesarean sections prior to 38 weeks gestation will also be recruited to the study. 15 – 30 women with normal pregnancies will also be recruited to the study to function as the control group.

There will be 4 categories of subjects:

A) Those at risk of respiratory morbidity at term

- Iatrogenic elective Caesarean section being performed < 38 gestational weeks in an otherwise well baby. This subgroup will be informative in relation to circulatory adaptation close to term.

B) Those at risk of pulmonary hypoplasia

- Patients with mid trimester PPROM
- Patients with persistent oligohydramnios of renal or nonrenal origin
- Patients whose fetuses have known: Congenital diaphragmatic hernia (CDH), Congenital cystic adenomatoid malformation (CCAM)

- Other space occupying lesions of the thorax (cardiomegaly, pleural effusion and skeletal dysplasia).

C) Those at risk of respiratory morbidity due to a cardiac cause
Women whose fetuses have a prenatal diagnosis of moderate/severe perimembranous ventricular septal defect (VSD)/atrioventricular septal defect (AVSD) in the absence of other structural heart disease including cases of Trisomy 21. The MH test may contribute to prediction of the need for neonatal intervention in this group.

D) Normal pregnancy control group

15.4 Demographic and baseline disease characteristics

Demographics
The following maternal details will be collected at enrolment:

Maternal age
Parity and Obstetric history
Weight and height (BMI calculation)
Socioeconomic grouping
Ethnicity
Smoking, alcohol and drug use
Medical history
Medication use
Antenatal administration of corticosteroids
Antenatal use of tocolytics
Gestational age at PPROM (weeks) if relevant
Interval from PPROM- delivery (days) if relevant

The following perinatal data will be collected:

Time interval between oxygen test and delivery
Fetal sex
Birth weight
Gestational age at delivery
Gestational age at maternal hyperoxygenation test
Indication for delivery

15.5 Efficacy analysis

Interim analysis will be performed monthly

15.5.1 Primary efficacy endpoint

Persistent pulmonary hypertension will be defined by echocardiography as well as clinical indicators as follows:
1) A requirement of at least 0.4 Fractional Inspired Oxygen to maintain a preductal saturation of ≥ 95%; and,

2) Normal Structural anatomy of the heart on echocardiogram; and,
3) In the presence of tricuspid regurgitant (TR) jet, an estimated right ventricular systolic pressure (using the Bernoulli Equation) ≥ 50% of the systemic systolic pressure measured at the start of the echocardiogram; or
4) In the presence of a patent ductus arteriosus (PDA of a low velocity shunt across the PDA from left to right such that the estimated Right Ventricular/ Pulmonary artery pressures was >50% systemic
5) In the absence of a TR jet or a PDA, an intraventricular septum bowing into the left ventricular cavity

15.5.2 Secondary efficacy endpoints

Severity of pulmonary hypertension
Composite of neonatal respiratory morbidity (Respiratory Distress Syndrome and Transient Tachypnea of the Newborn)
Neonatal intensive care unit (NICU) admission
28 day survival
Survival to discharge from hospital
Meconium Aspiration Syndrome
Umbilical Artery and vein pHs and base excess
Neonatal interventions required:

- Chest compressions
- Intubation
- Peak inspiratory pressure (PIP)
- Mean arterial pressure (MAP)
- Positive end expiratory pressure (PEEP)
- Adrenaline bolus at birth
- Hydrocortisone pre transfer
- Inotrope infusion pre transfer
- Nitric
- Surfactant
- High frequency oscillatory ventilation (HFOV) at birth
- Intravenous antibiotics
- Nitric oxide duration

15.6 Safety analysis

N/A

15.7 The level of statistical significance

We will accept a p value of < 0.05 as significant

15.8 Criteria for the termination of the trial

The end of trial will be the date of the last visit of the last subject.

15.9 Procedure for accounting for missing, unused and spurious data

Data will be stored for a maximum of 5 years and will be destroyed confidentially after the study has been completed and published. All spurious data will be destroyed.

15.10 Procedure for reporting any deviation(s) from the original statistical plan

Any deviations from the original statistical plan will be notified to the HPRA. Descriptive statistics will be used to summarise the findings of the study so deviations are unlikely to occur.

16 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

17 DATA HANDLING AND RECORD KEEPING

Both a hard copy and electronic versions of the data will be retained. Electronic data will be retained on a research computer database within the Rotunda Hospital. All data will be maintained on password protected computers and no patient data or names will be recorded.

17.1 Data collection, source documents and case report forms (CRF)

Source documents for this study will include hospital records and procedure reports and data collection forms. These documents will be used to enter data on the CRFs. All data entered on CRFs must be entered legibly. If an error is made, the error will be crossed through with a single line in such a way that the original entry can still be read. The correct entry will then be clearly inserted, and the alterations will be initialled and dated by the investigator.
Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained.
All documents will be stored safely in confidential conditions. On all study-specific documents other than the signed consent, the subject will be referred to by the study subject indenisation number/code.

17.2 Data reporting

The subjects will be identified by a study specific subjects number and/or code in the database. The name and any other identifying detail will not be included in any study data electronic file.

18 RETENTION OF ESSENTIAL DOCUMENTS

All records and documents will be maintained by the investigator for a period of at least 2 years after FDA/European Medicines Agency (EMA) approval of the medicinal product or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational medicinal product, whichever is longer.

19 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.
GCP monitoring will occur on site.

20 STUDY MONITORING

This study will be conducted in accordance with the latest approved protocol, ICH GCP, relevant regulations and SOP's. The sponsor will assign an independent monitor who will visit the investigator intermittently to validate compliance of the protocol to the GCP, the maintenance of the study related records, and the extensiveness and accuracy of a proportion of CRF entries compared to source data. The investigator will co-operate with the monitor to ensure that any potential discrepancies are resolved.
Monitoring procedures include a site initiation visit designed to clarity all prerequisites before the trial commences at the site, interim site monitoring visits and study close-out visits. The study will be monitored by regular scheduled visits to site and on-going communication via telephone and e-mail.
During site visits the monitor will review; original patient records for the patient group; CRFs; drug accountability records; investigator site file and document retention. Study procedures will be observed by the monitor and any issues will be discussed with the PI or designee as necessary.
All monitoring visits will be documented in a monitoring visit report and these reports will be filed in the Trial Master File.
At a minimum, source documentation will be available to substantiate subject identification, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of AEs; administration of concomitant medication; drug receipt/dispensing/return records; study drug administration information; and dates of subject completion, discontinuation from treatment, or withdrawal from the study, including the reason if appropriate.
CRF entries will be verified with the source documentation, if applicable (in some cases there are no source pages, therefore verification is not necessary). If any data, signatures, or forms are missing or incorrect, the Investigator or designee will be informed and corrections will be made. Direct access to all source documents must be guaranteed by the PI, who must provide support at all times for these activities.

21 AUDITS AND INSPECTIONS

This trial may be subject to internal or external auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents will be given at that time.

22 ETHICS

Approval will be gained from our local ethics committee in the hospital.

22.1 Declaration of Helsinki

The sponsor will ensure that this study is conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

22.2 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and 2005/28/EC.

22.3 Approvals

Required documents including the protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other required documents will be submitted to a recognised research ethics committee and the competent authority for written approval.
The sponsor will submit and obtain approval from the above parties for substantial amendments to the original approved documents.

22.4 Informed consent

Informed consent will be taken by the study investigators, Professor Fionnuala Breathnach and/or Dr Ann McHugh. Consent will be taken at the subject's recruitment visit to the ultrasound department following recruitment, when they attend for the hyperoxygenation test. The subject will have received an information leaflet and the consent form prior to attending the ultrasound department giving them adequate time to make an informed decision on partaking in the study and to give informed consent. Professor Fionnuala Breathnach and/or Dr Ann McHugh will explain the nature of the study to the subject, and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subject, the person who administered the informed consent form.

22. 5 Benefits and risks assessment

Persistent pulmonary hypertension of the newborn (PPHN) occurs in 0.5 to 7 per 1000 live births. It results in a mortality ranging between 4 to 33% [1-2]. Some degree of pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure. Increased pulmonary vascular resistance in the newborn will produce extrapulmonary shunting of blood which can lead to severe and potentially unresponsive hypoxemia and significant morbidity and mortality. Pulmonary hypertension typically accompanies pulmonary hypoplasia when diminished surface area for gas exchange and inadequate pulmonary blood flow lead to hypoxia and remodelling of the resistance pulmonary arterioles[3] The ability to predict the

occurrence and severity of fetal pulmonary hypoplasia antenatally, would facilitate optimal antenatal and neonatal management.

Recent studies have shown that fetal pulmonary vasculature reacts to maternal hyperoxygenation [5-7]. Following maternal oxygen therapy, a decrease in the pulmonary vascular resistance as demonstrated by pulmonary venous Doppler, is deemed to indicate vasoreactivity in the pulmonary vascular bed. Small studies to date indicate that a lack of vasoreactivity in response to maternal hyperoxygenation may serve as a useful clinical tool in predicting lethal pulmonary hypoplasia in those at- risk fetuses. The measurement of peripheral pulmonary velocity waveforms before and after maternal hyperoxygenation may therefore help in determining the risk of developing pulmonary hypertension

We do not foresee any undue risk to the subjects. Oxygen is safe to use in pregnancy and is not known to cause any adverse maternal of fetal effects. It will be administered for a total duration of 10 minutes so the side effect of epistaxis as described with chronic oxygen use is unlikely to occur.
Participation in this study is not likely to be associated with any benefit to mother or baby. The information from this study is generated for the purpose of the pilot study only. It will be analysed at a later date by the research team to assess whether the information from the pilot study would justify a larger research study investigating the feasibility of using the hyperoxygenation test to predict the occurrence and severity of PPHN in the future. This test may eventually become part of the routine assessment of foetuses at risk of PPHN during the antenatal period.
It is expected that if the patient is scheduled to have a regular fetal growth scan at the same gestation as the hyperoxygenation study scan, both assessments can be done at the same examination, to facilitate patient hospital attendances.
The risks associated with participation in the study can be considered negligible and the burden can be considered minimal

22.6 Subject confidentiality

The trial staff will ensure that the subjects' anonymity is maintained. The subjects will be identified only by initials and a subject's identification number on the CRF and any database. All documents will be stored securely. The study will comply with the Data Protection Act.

22.7 Other ethical considerations

N/A

23 FINANCING AND INSURANCE/INDEMNITY

The Rotunda Hospital holds Public Liability ('negligent harm') and Clinical Trial ('non-negligent harm') insurance policies which apply to this trial.

Friends of the Rotunda is funding this trial.

Dr Ann McHugh's salary is funded by the Royal College of Surgeons in Ireland.

24 CLINICAL STUDY REPORT AND PUBLICATION POLICY

Study investigators will sign the clinical study report. All original data and its analysis will be required to be stored until all relevant intellectual property assignments are made including relevant research publications and a PhD.

25 REFERENCES

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HPRA
An tÚdarás Rialála Táirgí Sláinte
Health Products Regulatory Authority

7th October 2016

Royal College of Surgeons in Ireland,
RCSI Education & Research Centre,
Beaumont Hospital,
Beaumont,
Dublin 9.

European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations, 2004

Re: CT Number: CT 900/594/1 - Oxygen
Case number: 2181617
EudraCT number: 2016-003181-12
Protocol number: HOTPOT1
Title of trial: Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension?

Dear Sir/Madam,

The Health Products Regulatory Authority has considered the application dated 9th August 2016 seeking authorisation to conduct the above clinical trial.

On the basis of the evidence available, the application is acceptable.

Please note that the date of this letter is the date of authorisation of the trial.

In accordance with Article 11 of Directive 2001/20/EC, confirmation of the authorisation of a clinical trial is mandatory for the updating of EudraCT, the EU database for clinical trials, and will be made public. Therefore, the Health Products Regulatory Authority requires that you provide the following information for this clinical trial as soon as it is available:

- Name of the responsible ethics committee
- Ethics committee opinion (favourable, not favourable, withdrawal)
- Date of the ethics committee opinion

If any changes are made to the EudraCT application form, you are reminded to provide the latest version of the XML file to the Health Products Regulatory Authority for uploading to the EudraCT database. If in future, the XML file is updated as a result of a non-substantial amendment please submit the revised version of the XML file with the documentation for the next substantial amendment application.

Yours sincerely,

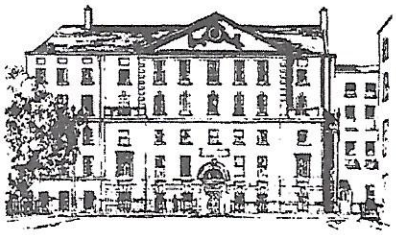
Kinga Wilczyńska

Digitally signed by Kinga Wilczyńska
DN: cn=Kinga Wilczyńska, o=Health Products Regulatory Authority, ou=Business Process Co-Ordination Unit, email=kinga.wilczynska@hpra.ie, c=IE
Date: 2016.10.10 16:24:36 +01'00'

A person authorised in that behalf by the Board of the said Authority

An tOspidéal Náisiúnta Máithreachais
The National Maternity Hospital
Founded in 1894

Sráid Holles, Baile Átha Cliath 2 • Holles Street, Dublin 2.
Telephone: (01) 6373100. Fax: 6766623. Web: www.nmh.ie



Máistir/ Master: Dr. Rhona Mahony

PRIVATE AND CONFIDENTIAL

Professor Fionnuala Breathnach,
Associate Professor Obstetrics and Gynaecology,
Royal College of Surgeons in Ireland,
Rotunda Hospital,
Parnel Square,
Dublin 1.

13th September 2016

Our ref: **EC 28.2016**

Re: **Can sonographic assessment of pulmonary vascular reactivity following the maternal hyperoxygenation test predict neonatal outcome in foetuses at risk of pulmonary hypertension?**

Dear Professor Breathnach,

The above study was approved by the ethics committee on the 05th September 2016.

We wish you success with the study.

Kind regards,
Yours sincerely,

Dr. John Murphy
Chairman,
Ethics Research Committee

Cc. Dr. Ann McHugh, Specialist Registrar in Obstetrics and Gynaecology, Royal College of Surgeons in Ireland, Master's House, Rotunda Hospital, Parnell Square, Dublin 1.

Paola della Porta PhD
Associate Director of Research
Office of Research and Innovation
Royal College of Surgeons in Ireland
111 St. Stephens Green, Dublin 2, IRELAND
E-mail: pdellaporta@rcsi.ie Tel. 00-353-1-4022393

Receipts and Validation section
Health Products Regulatory Authority
Kevin O'Malley House
Earlsfort Centre
Earlsfort Terrace
Dublin 2

02/09/2016

Title of Trial: HOTPOT
Principle Investigator: Fionnuala Breathnach
EudraCT No.: 2016-003181-12

To whom it may concern

This letter confirms that RCSI has agreed to sponsor the above referenced investigator-led clinical trial.

Sponsorship is subject (but not limited) to approval from the Research Ethics Committee and HPRA.

Signed on behalf of RCSI

Paola della Porta

Paola della Porta
Associate Director of Research, RCSI

HDI

CERTIFICATE OF INSURANCE

This is to certify that a Policy of Insurance as described below has been issued to the Insured

Policy Number:	390-01159615-14010
The Insured:	RCSI Education & Research Centre
Period of Insurance:	From 1st December 2017) Local Standard Time at the to 30th November 2018) Address of the Insured both days inclusive
Cover:	Clinical Trial Insurance Specific Trial Policy in accordance with ABPI guidelines
Business:	The undertaking of a Trial by or on behalf of the Insured in connection with Protocol No HOTPOT - Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict fetal outcome in fetuses at risk of pulmonary hypoplasia?
Limits of Indemnity:	EUR 6,500,000 in the aggregate for the Period of Insurance inclusive of Legal Costs
Patients Number:	60
Territory:	The Republic Of Ireland
Excess:	Nil
Retroactive Date:	29th October 2004 Local Standard Time at the address of theInsured

Nothing contained in this Certificate shall in any way be held or construed to vary alter or waive any of the terms conditions or provisions of the Policy

This Certificate is only a summary of the Policy Reference should be made to the Policy for the full terms conditions and exceptions

Date: 05 December 2017

Signed
For and on behalf of
HDI Global SE Company -UK Branch

HDI

CERTIFICATE OF INSURANCE

This is to certify that a Policy of Insurance as described below has been issued to the Insured

Policy Number:	390-01159615-14010
The Insured:	Clinical Research Centre of the Royal College of Surgeons in Ireland
Period of Insurance:	From 1st December 2016) Local Standard Time at the to 30th November 2017) Address of the Insured both days inclusive
Cover:	Clinical Trial Insurance Specific Trial Policy in accordance with ABPI guidelines
Business:	The undertaking of a Trial by or on behalf of the Insured in connection with Protocol No HOTPOT - Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict fetal outcome in fetuses at risk of pulmonary hypoplasia?
Limits of Indemnity:	EUR 6,500,000 in the aggregate for the Period of Insurance inclusive of Legal Costs
Patients Number:	60
Territory:	The Republic Of Ireland
Excess:	Nil
Retroactive Date:	29th October 2004 Local Standard Time at the address of theInsured

Nothing contained in this Certificate shall in any way be held or construed to vary alter or waive any of the terms conditions or provisions of the Policy

This Certificate is only a summary of the Policy Reference should be made to the Policy for the full terms conditions and exceptions

Date: 24 January 2017

Signed
For and on behalf of
HDI Global SE Company -UK Branch

The Rotunda Foundation

5th December 2016

Professor Fionnuala Breathnach
RCSI Rotunda Hospital
Parnell Square
Dublin 1

Re: The Rotunda Foundation (RF) Grant Award 2016 (Reference: RF/HOTPOT/2016)

Dear Professor Breathnach,

The Rotunda Foundation Charity (CHY20091) has noted that your research proposal has received full ethics approval and therefore, approval for a grant application can proceed to fund the following research project:

Research Project Title:

Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict fetal outcome in fetuses at risk of pulmonary hypoplasia?

Principal Investigator:	Professor Fionnuala Breathnach
Lead Researcher:	*Dr. Ann McHugh (PhD Candidate)*
Co-PLS:	*Dr. Afif EL-Khuffash & Dr. Orla Franklin*

The total grant awarded by **the Rotunda Foundation** is **€26,500** to cover costs as detailed in the table below:-

Budget Resources	**2016**
- Oxygen Cylinder (Compressed Gas) Given at 10 Litres /minute x 10 minutes 100 Litres x 30 Patients Total 3,000 Litres of Oxygen Small Cylinder €9.46 with Connector	Consumable
- NICOM Stickers 100e per packet (4 Stickers) 1 box = 25 packets (2,500e) Requirements – 2 boxes Total: €5,000	Consumable
- Oxygen Face Mask (Non-rebreather Type) Total: 40	Consumable
- Blood Vacuettes Total: 40	Consumable

- BNP Neonatal Blood Bottles Total: 40	Consumable
➢ **Total of Consumables**	**€6,500**
➢ **Monitoring / Pharmacovigilance Costs** (Provided by RCSI CRF who will act as Sponsor)	**€5,000**
➢ **ECRF /Database Compliant for HPRA Safety Reporting**	**€2,000**
➢ **Research Assistant (0.3 WTE)**	**€9,000**
➢ **Overhead to Rotunda Research Department**	**€2,000**
➢ **Travel**	**€2,000**
Total Grant Awarded - **5th December 2016** - **Cheque 504598**	**€26,500**

Reporting:

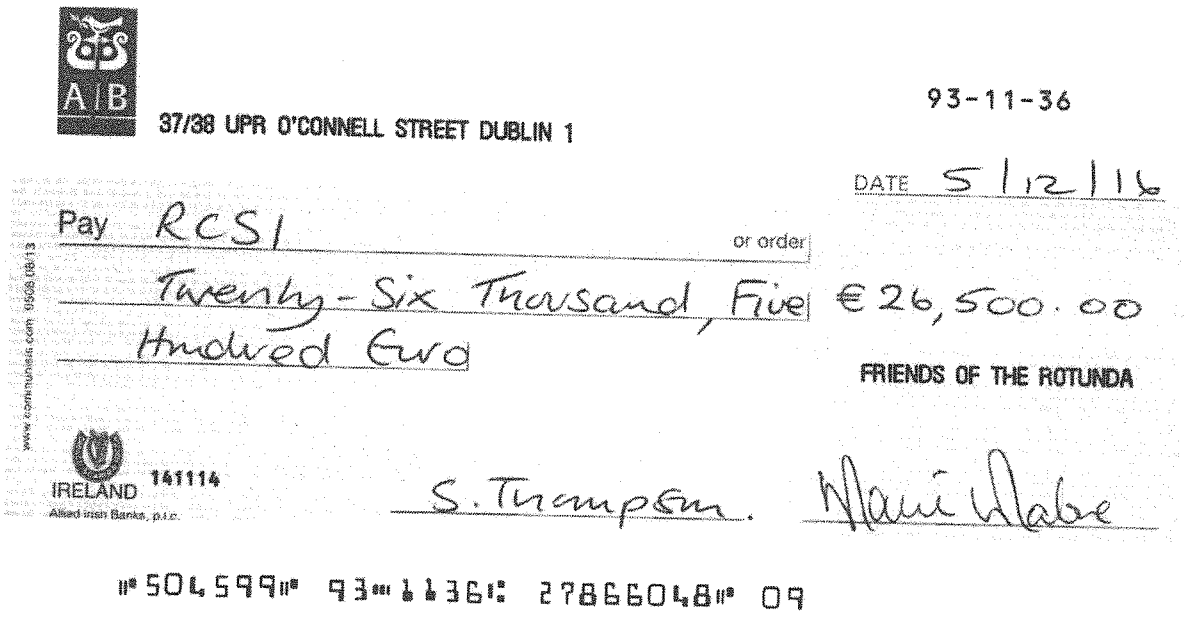
The Lead Investigator is required to submit a progress report and a financial statement of expenditure to the Research Review Panel of The Rotunda Foundation at the end of a twelve month period from the commencement of your study and annually thereafter, if applicable.

Please also notify The Rotunda Foundation's administration when and where you publish and/or present your results. The Rotunda Foundation's logo should be included on any publications or presentations with a notice advising that seed funding was awarded.

Please ensure that you keep us advised of any change that may affect this research study.

Your acceptance of this award has been acknowledged. Please do not hesitate to contact me should you require any further information.

Yours sincerely,

S. Thompson.

Sheila Thompson
The Rotunda Foundation