1 TITLE PAGE

Study title:

Name of Test Drug: Oxygen

Indication studied: Predication of PPHN

Study description: Sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy in the prediction of neonatal outcomes in fetuses at risk of pulmonary hypertension

Sponsor: Royal College of Surgeons Ireland (RCSI)

Protocol: EudraCT # 2016-003181-12

Clinical Phase: Phase 4

Study dates: First patient first visit: 06-Feb-2017
Last patient last visit: 22-Jul-2018

Investigators: Chief Investigator: Prof Fionnuala Breathnach
Address:
Rotunda Hospital,
Parnell St
Dublin 1

Sub-Investigator: Dr Ann Mc Hugh
Address:
Rotunda Hospital
Parnell St
Dublin 1

Sponsor signatory:	Muiris Dowling, RCSI Sponsor Officer
GCP Statement:	This study was performed in compliance with ICH Good Clinical Practice (GCP) including the archiving of essential documents
Date of report:	14-Feb-2020

2 SYNOPSIS

NAME OF SPONSOR:	RCSI
NAME OF FINISHED PRODUCT:	N/A
NAME OF ACTIVE INGREDIENT(S)	Oxygen
Title of Study	Can sonographic assessment of pulmonary vascular reactivity following maternal hyperoxygenation therapy predict neonatal outcome in fetuses at risk of pulmonary hypertension?
Phase:	4
Publication	1.McHugh A, El-Khuffash A, Bussmann N, Doherty A, Franklin O, Breathnach F. Hyperoxygenation in pregnancy exerts a more profound effect on cardiovascular hemodynamics than is observed in the nonpregnant state. Am J Obstet Gynecol. 2019;220(4):397.e1-.e8.
Study centre(s)	Rotunda Hospital
Study period	06-Feb-2017 to 22-Jul-2018
Date of final analysis	13-Feb-2020
Objectives	**Primary Objective** The main objectives of the study were 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.

	Secondary Objective 1. Predict neonatal survival and pulmonary hypertension by measurement of PA reactivity to MH in fetuses at risk of neonatal respiratory morbidity. 2. Assess serial changes in cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR) before, during and after MH.
Methodology	This was a prospective cohort study. It evaluated 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 severity of pulmonary hypertension in the neonate was formally assessed with a neonatal echocardiogram performed within the first 48 hours of life. The Study was undertaken in the Rotunda Hospital, Dublin.

Number of patients	Planned: 60-80 Number of subjects recruited and analysed: 66
Main criteria for inclusion	The inclusion criteria can be divided into 5 main categories: **A) <u>Those at risk of respiratory morbidity at term</u>** • Iatrogenic elective LSCS being performed <38 weeks gestation 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, hydrops and skeletal dysplasia). **C) <u>Those at risk of respiratory morbidity due to a cardiac cause</u>** • Women with a prenatal diagnosis of moderate/ severe perimembranous ventricular septal defect (VSD)/ atrioventricular septal defect (AVSD) in the fetus, in the absence of other structural heart disease including cases of Trisomy 21. MH test may contribute to prediction of the need for neonatal intervention in this group. **D) Normal gestation-matched uncomplicated singleton pregnancies to serve as a control group.** **E) Non-pregnant control group**
Test product, dose and mode of administration	Medical oxygen, (O2 100% v/v inhalational gas), administered to the subject via a non-rebreather face mask at a rate of 8-12 litres per minute with the patient lying in a semi recumbent position.
Duration of treatment	Ten minutes
Criteria for evaluation	Fetal Doppler studies. 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

Statistical methods	SPSS 24.0

SUMMARY CONCLUSIONS	
Efficacy Results	Hyperoxygenation during the third trimester is associated with a fall in maternal cardiac index and a rise in systemic vascular resistance without recovery to baseline levels at 10 minutes after cessation of hyperoxygenation. The hemodynamic changes that were observed in this study in response to hyperoxygenation therapy during pregnancy could counteract any intended increase in oxygen delivery. Our findings indicate a reduction in fetal pulmonary vascular resistance with a resultant increase in fetal pulmonary blood flow. 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
Safety Results:	No adverse side effects of oxygen administration reported
Conclusion	The observed maternal effects of hyperoxygenation call for a reevaluation of the role of hyperoxygenation treatment in the nonhypoxemic pregnant patient. Maternal hyperoxygenation offers the opportunity to assess the reactivity of the pulmonary vasculature before birth. . An increase in left ventricular cardiac output and ejection fraction in the neonates who demonstrated a prenatal response to hyperoxia, suggests that the hyperoxygenation test can inform us of functional rather than anatomical information in relation to the pulmonary arteries in utero.
Date of the Report	14/02/2020

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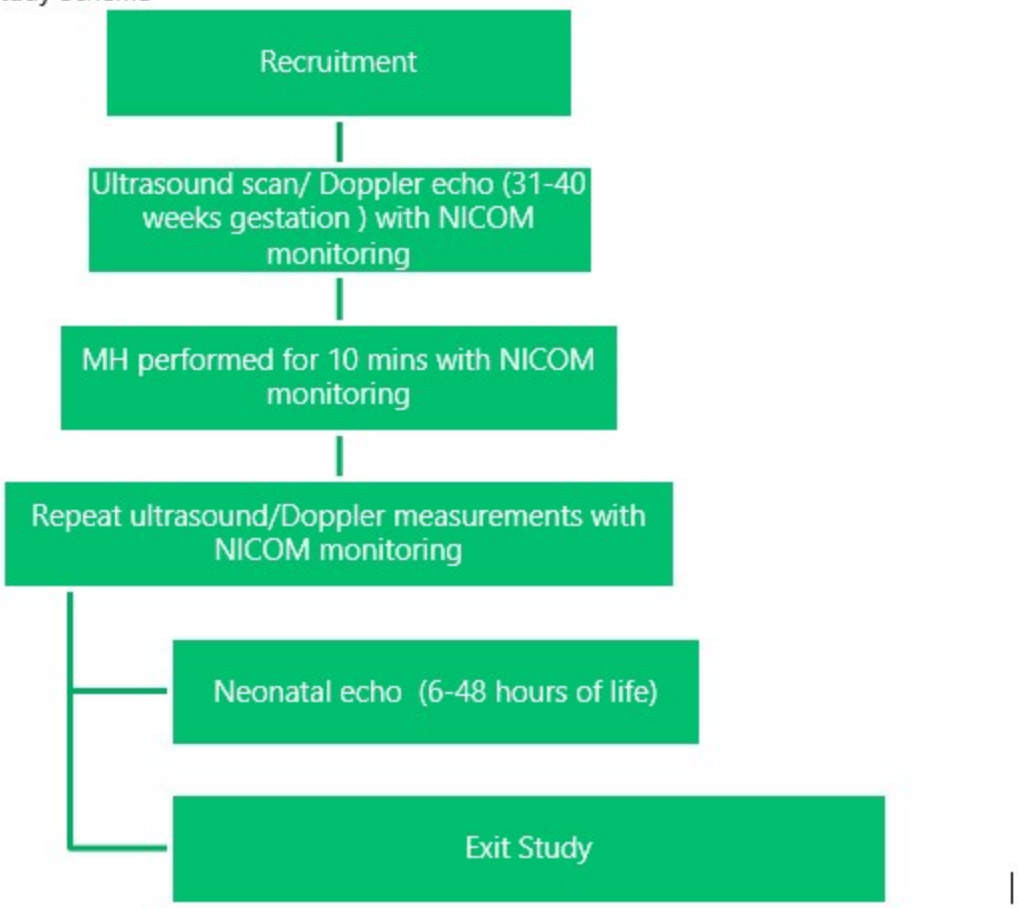
Figure 1: Study Schema



- Test period – 30 minutes total
- NICOM monitoring period 30 minutes (10 minutes pre MH, 10 minutes during MH and 10 minutes after MH)

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STATISTICAL AND ANALYTICAL PLANS 30

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). 30

Analysing Haemodynamics 30

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. 30

DETERMINATION OF SAMPLE SIZE 31

Sample sizes of between 24 (12 per group) and 50 have been recommended variously for pilot studies Following these broad recommendations and that of the NHS 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 AUT-F0316-2 47/57 [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-aFeasibility-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 2 groups (responders and non repsonders) were chosen. 31

The number of subjects reported to be in the baseline period are not the same as the worldwide number enrolled in the trial. It is expected that these numbers will be the same. 31

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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]. 35
12 35

Table 1: Demographics

Baseline characteristics (n=46)	
Mean Age (years)	31.7 (SD 7.1)
Median BMI (Kg/m²)	29.6 [IQR 27.6-33.5]
Median Gestation age (weeks)	35.0 [IQR 33-37]
Primiparous	15 (33%)
Caucasian	39 (85%)
Hb level third trimester (g/dL)	11.7 (11.1-12.7)

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4 ETHICS AND REGULATORY APPROVAL

4.1 INDEPENDENT ETHICS COMMITTEE APPROVAL

The study protocol and all its amendments, and the patient information sheet(s) were reviewed and approved by the appropriate independent ethics committees as detailed in table one below. A copy of the initial ethics approval can be found in Appendix 2

4.2 ETHICAL CONDUCT OF THE STUDY

The study was performed in accordance with the current version of the declaration of Helsinki (2013). The study was conducted in compliance with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP)

4.3 PATIENT INFORMATION AND CONSENT

All patients provided written informed consent to participate in the study prior to screening.

The patient information leaflet detailed the procedures involved in the study (aims, methodology, potential risks, and anticipated benefits) and the investigator explained these to each patient. The patient was then given adequate time to consider the information before signing and dating the informed consent form to indicate that they fully understood the information, and willingly volunteered to participate in the study. The patient was given a copy of the patient information leaflet and informed consent form for their information and a copy was filed in the patient medical records. The original copy of the informed consent form was filed in the investigator site file (ISF). A

sample of the patient information sheet and informed consent form can be found at **Appendix 4**

4.4 REGULATORY APPROVAL

The study gained full regulatory approval from the Health Products Regulatory Agency (HPRA) on 07-Oct-2016 and was issued with the following CT reference number: CT 900/594/1.

A copy of the initial HPRA approval can be found in **Appendix 3**

5 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Table II shows the key study personnel involved in the trial.

Table II: key study personnel

Title	Name and affiliation
Chief Investigator	Prof. Fionnuala Breathnach, Department of Obstetrics & Gynaecology, RCSI Rotunda
Lead Investigator	Dr Ann Mc Hugh, Department of Obstetrics & Gynaecology, RCSI Rotunda
Sponsor	Royal College of Surgeons in Ireland
Chief Pharmacist	Dr Brian Cleary
Statistician	Dr Ann Mc Hugh

6 INTRODUCTION

6.1 THERAPEUTIC AREA

In utero, the placenta functions as the organ for gaseous exchange (1). Pulmonary hypertension is a normal state for the fetus and pulmonary vascular tone increases with advancing gestational age (2). 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 (3). Fetal pulmonary arterial vascular impedance decreases during the second half of pregnancy until 34 to 35 weeks gestational age (4). Despite ongoing lung growth after 34 to 35 weeks gestation, the pulmonary vascular impedance thereafter, remains unchanged (5). At term, pulmonary blood flow increases substantially to almost half of the combined ventricular output (6). A low oxygen tension environment exists in utero, which promotes high intrinsic myogenic tone and high vasocontractility (7). At birth, there is a reduction in pulmonary arterial pressure and resistance, due to an increase in oxygen tension and up to a ten-fold rise in pulmonary blood flow (7). Neonatal survival is dependent upon a rapid, complex and well-orchestrated transition from the intra- to extrauterine environment (8). Normal transition to newborn circulation requires high fetal pressures to fall, with dilatation of the pulmonary vessels. Previous studies have indicated that the capacity of pulmonary arteries to dilate can be judged prenatally, by administering high-dose oxygen to the mother (9-11).

Hyperoxygenation

Studies have shown that fetal pulmonary vasculature reacts to maternal hyperoxygenation (MH) (5, 12, 13). Oxygen induces the release of several vasodilators including endothelium-derived nitric oxide and prostacyclin (PGI2), resulting in a decrease in pulmonary vascular resistance and an increase in pulmonary blood flow (14). Following maternal oxygen administration, a decrease in the pulmonary vascular resistance, as demonstrated by the pulmonary artery doppler, is deemed to indicate vasoreactivity in the pulmonary vascular bed (15). Studies 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 (16, 17). The increase in fetal pulmonary blood flow following MH results in increased venous return to the left heart and this response increases with gestational age (18). The measurement of pulmonary velocity waveforms before and after MH may therefore help in determining how the fetus will adapt to the extra-uterine environment and transition to neonatal life.

Sonographic assessment

Several methods have been proposed to assess fetal pulmonary vascular development. These include measurement of the fetal chest circumference, chest area, chest area minus heart area,

ratio of the 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 (10). Some of these methods are time consuming to perform and there can be considerable variability in measurements between different sonographers (19). 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 (IUGR). There are

limitations to using many of these techniques. For example, the chest circumference to abdominal circumference ratio cannot be used in fetuses with large chest circumferences, therefore fetuses with polycystic kidneys, obstructive uropathy or omphalocele would be excluded. The reactivity of the pulmonary artery to changes in fetal oxygen tension can be detected by noninvasive doppler ultrasound techniques between 31 and 36 weeks of gestational age (5). Normative curves for the development of pulmonary reactivity induced by hyperoxia during gestation have been established (14). The ability to predict the fetal transition to neonatal life by a method that is non-invasive and reproducible would be beneficial for the obstetric management, for parental counselling and for determining optimal perinatal management. Approximately ten

percent of neonates will require some form of clinical intervention at birth with one percent requiring more extensive resuscitation (20). If 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 (2). This results in a mortality ranging between 4 to 33% (21, 22) . Some degree of pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure (3). Increased pulmonary vascular resistance in the newborn will produce extrapulmonary shunting of blood which can lead to severe and potentially unresponsive hypoxemia (3). It is vitally important for clinicians to have an understanding of the changes that occur during the transition to neonatal life and to predict which neonates may have difficulty transitioning (20). Prediction of neonatal pulmonary hypertension may influence the delivery planning for particularly high risk cases.

7.2 RATIONALE FOR THE STUDY

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.

The hemodynamic effects of oxygen therapy are under-recognized and the impact of hyperoxygenation on maternal hemodynamics is currently unknown. Using noninvasive cardiac output monitoring which employs transthoracic bioreactance, we examined the effect of brief hyperoxygenation on cardiac index, systemic vascular resistance, blood pressure, stroke volume, and heart rate in pregnant mothers during the third trimester, compared with those effects observed in a nonpregnant population subjected to the same period of hyperoxygenation.

8 STUDY OBJECTIVES

Primary Objectives

Primary Objective

The main objectives of the study were as follows:

Assess vasoreactive response to maternal hyperoxygenation (MH) in utero.

Assess the ability of MH to predict the degree of pulmonary vasculopathy present prior to birth.

Evaluate if pulmonary artery (PA) reactivity to MH identifies fetuses that will develop pulmonary hypertension.

Secondary Objective

Predict neonatal survival and pulmonary hypertension by measurement of PA reactivity to MH in fetuses at risk of neonatal respiratory morbidity.

Assess serial changes in cardiac output (CO), stroke volume (SV) and systemic vascular resistance (SVR) before, during and after MH.

9 STUDY DESIGN

9.1 OVERALL STUDY DESIGN AND PLAN

Methods

Overview

In the human fetus, blood flow velocity waveforms can be recorded from the right and left pulmonary arteries (PA) 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 (23). Doppler examination of blood flow in the main stem of the fetal pulmonary arteries fetus is feasible, and increases our insight into the lung perfusion of the human fetus. Mean decreases in PA PI following MH of between 18.0 and 21.2% have been previously described in normal fetuses (5). A cut off level of ≥20% decrease in the PA PI from the baseline have been studied and deemed to demonstrate pulmonary reactivity (5, 16). However, there remains large individual variability (24, 25). In one study of normal fetuses, nearly one-third had a decrease in PA PI that was less than 20% after an initial positive oxygenation test earlier in gestation (14). Values for reactivity in our study accounted for this variability, and were based on a previous study where a decrease in PA PI of ≥10% from the baseline level was used to characterize a reactive test or positive responder (17).

Study Procedure

All participants underwent a standard ultrasound examination for estimation of fetal weight, amniotic fluid volume measurements, fetal heart rate and umbilical and middle cerebral artery doppler investigations. Image-directed pulsed and color Doppler equipment (Voluson E8) was used with a 5-MHz sector probe. A fetal echocardiogram was performed according to an

agreed protocol on fetuses between 31 and 40 weeks completed gestation. This involved a sequential segmental analysis of the atria, ventricles, and great arteries and their connections. Specific echocardiographic Doppler studies included those of the proximal main PA and ductus arteriosus. All Doppler recordings were obtained using the lowest high-pass filter level (100 Hz), and the spatial peak temporal average power output for color and pulsed Doppler was kept at <100 mW/cm (26). An angle of ≤15° between the vessel being studied and the Doppler beam was deemed acceptable and used for analysis. The following measurements specific to the pulmonary artery Doppler waveform were recorded: The peak systolic velocity (PSV), end diastolic velocity (EDV), time-averaged velocity (TAV), pulsatility index (PI), resistance index (RI), ejection time (ET) and acceleration time (AT). The ductus arteriosus waveform was obtained in the traditional longitudinal ductal arch view. Reported values were averaged from three consecutive waveforms.

Oxygen was administered to the patients while in a semi-recumbent position. Oxygen was administered at a rate of 10 litres per minute (L/min) for a duration of 10 minutes via a non-rebreather mask. Following MH, a repeat fetal echocardiogram was performed and all Doppler recordings were repeated. Each fetus served as its own control. Recordings were stored on the ultrasound machine for further analysis and for data safety monitoring purposes. The

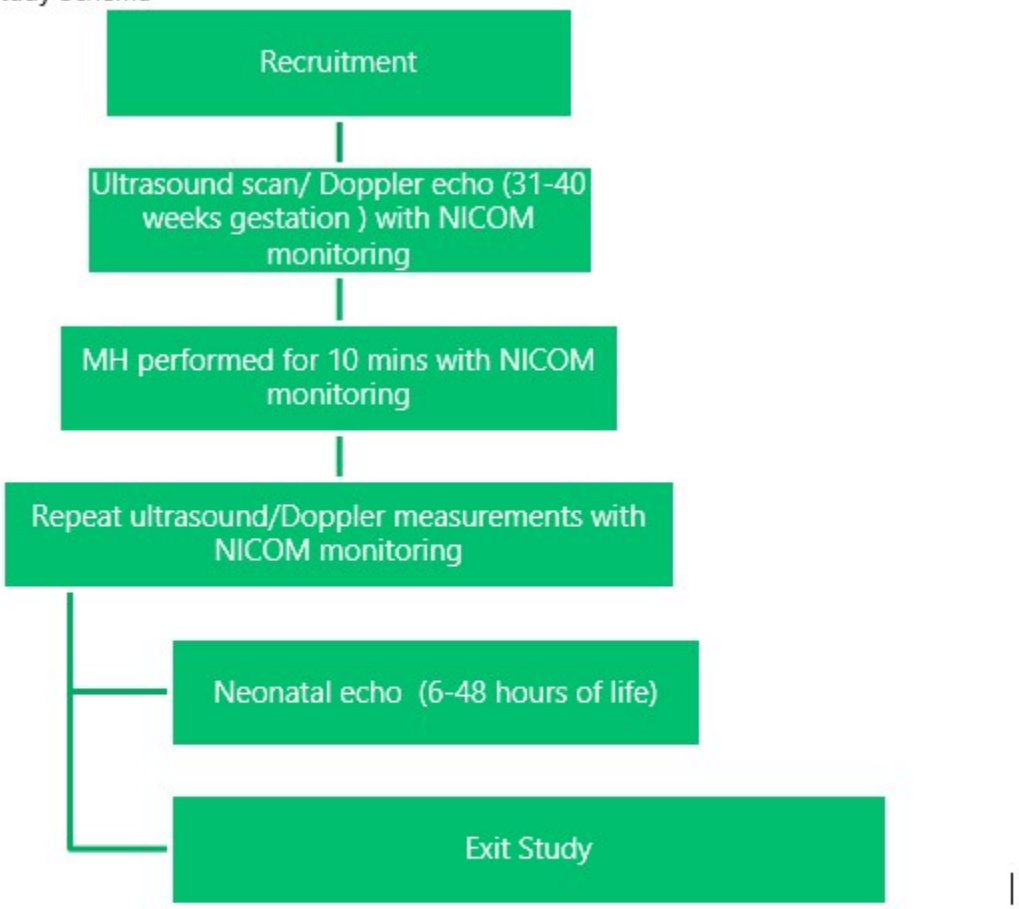
hyperoxygenation test was considered positive when the PI of the fetal PA decreased by ≥10% from its baseline (responders). Where the fetal PA PI did not decrease by at least 10%, cases were classified as non-responders.

Hemodynamic monitoring was performed in a continuous manner over a 30-minute period using noninvasive cardiac output monitoring. Hyperoxygenation (O_2 100% v/v inhalational gas) was carried out at a rate of 12 L/min via a partial non-rebreather mask for 10-minutes. Cardiac index, systemic vascular resistance, stroke volume, heart rate, and blood pressure were recorded before hyperoxygenation, at completion of hyperoxygenation, and 10 minutes after the cessation of hyperoxygenation. Two-way analysis of variance with repeated measures was used to assess the change in hemodynamic indices over time and the differences between the 2 groups.

STUDY TIMING

Figure I **Schematic diagram demonstrating study design**

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)

STUDY LOCATION

Rotunda Hospital, Dublin, Ireland

9.2 SELECTION OF STUDY POPULATION

INCLUSION CRITERIA

Patients fulfilling the following criteria were eligible for inclusion in the study: Overall description of trial subjects

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

The following subgroups of patients recruited to the study, all of whom carry a fetus at risk for PPHN:

A) Women who are carrying a fetus at risk of pulmonary hypoplasia:
   a. Mid-trimester PPROM
   b. Congenital diaphragmatic hernia
   c. 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) In addition, a group of 20 gestation-matched uncomplicated singleton pregnancies will be recruited to serve as a control group.

E) Non-pregnant group

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

EXCLUSION CRITERIA

Patients fulfilling the following criteria were excluded from participation in the study:

- 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
- 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

WITHDRAWAL OF PATIENTS FROM THERAPY OR ASSESSMENT

Patients were free to withdraw from the study at any time without giving a reason. Patients were advised that if they requested to withdraw from the study, at any time during the trial, then this would have no negative consequences on further care

DESCRIPTION OF INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

The investigational medicinal product (IMP) was Oxygen

1.1 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 was 60% FiO2 administered via a face mask over ten minutes.
This dosing has been used in numerous studies previously without any adverse effects.

1.2 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: 8-12Litres/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

Oxygen was stored in appropriate oxygen cylinders

1.3 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.

1.4 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.

1.5 Prior and concomitant therapy

Any medication, other than the study medication taken during the study was recorded in the CRF.

1.5.1 Prohibited medications

Bleomycin and amiodarone.

9.3 EFFICACY AND SAFETY VARIABLES

EFFICACY AND SAFETY MEASUREMENTS ASSESSED

Table 3 shows the schedule of assessments and procedures.

Table 3 Schedule of assessments and procedures

12.3 Study assessments and procedures

Figure 2: Schedule of events

Procedures	Visit 1 Screen /Recruit	Visit 2 24-40 weeks gestation	Visit 3	Visit 4
Inclusion/Exclusion Criteria	x	X		
Informed consent		X		
Medical history		X		
Physical examination and weight/height		X		
Vital signs		X		
Concomitant medications		X		
Ultrasound scan and Doppler echo		X		
Hyperoxygenation for 10 minutes 60% FiO2		X		
Repeat ultrasound and Doppler echo		X		
NICOM assessment over the 30minutes of testing		X		
Neonatal weight/demographics			X	
Neonatal echo (6-48hours of life)				X

Endpoint Assessments

Forty-six pregnant and 20 non-pregnant women with a median age of 33 years (interquartile range, 26-38 years) and 32 years (interquartile range, 28-37 years) were recruited prospectively, respectively (P=.82). The median gestational age was 35 weeks (33-37 weeks). In the pregnant group, there was a fall in cardiac index during the hyperoxygenation exposure period (P=.009) coupled with a rise in systemic vascular resistance with no recovery at 10 minutes after cessation of hyperoxygenation (P=.02). Heart rate decreased after hyperoxygenation exposure and returned to baseline by 10 minutes after cessation of therapy. There was a decrease in stroke volume over the exposure period, with no change in systolic or diastolic blood pressure. In the non-pregnant group, there was no significant change in the cardiac index, systemic vascular resistance, stroke volume, heart rate, or systolic or diastolic blood pressure during the course of exposure to hyperoxygenation.

Each fetus was appropriately grown for gestational age (all between the 10th and 90th percentile growth curve). The mean estimated fetal weight was 2660g +/- 626g at the time of the sonographic assessment. In all cases, amniotic fluid volumes based on a single deepest vertical pool were within the normal range (4.8cm +/- 1.8).

Successful acquisition of pulmonary artery indices was achieved in all participants. A decrease in fetal PA PI was observed following maternal hyperoxygenation, with a mean decrease of 21% [9-36] from the baseline. The resistance index of the PA decreased following MH (Table 2). There was an increase in PA AT leading to an increase in AT: ET, indicating a fall in pulmonary vascular resistance, following MH (Figure 1). No changes were observed in the pulsatility indices of the UAD or MCA following hyperoxygenation (0.96 to 0.99, p=0.95 and 1.70 to 1.72, p=0.98), respectively. There were no significant changes in the peak systolic, end-diastolic, mean velocities or resistance indices across the DA following MH. Fetal heart rate did not change significantly in response to MH (pre MH 140 ± 11.7, Post MH 136.8 ± 7.7, p=0.08). There was a significant increase in MCA blood flow, but not in MCA resistance indices.

The Caesarean delivery rate in this cohort was 54.3% (25/46).A physical examination at birth was recorded as normal for all newborn subjects. A neonatal echocardiogram was performed on 71.7% (n=33) of recruited cases. Neonates were divided into those that responded to MH in utero (responders) and those that did not respond (non-responders). There was no difference in mean gestational age at delivery or in mean birthweight between the two groups (Table 3). Fetuses that responded to MH (a decrease PA PI >10% from baseline) were more likely to have a higher left ventricular cardiac output (135 ± 25 verses 111 ± 21 mL/Kg/min, p=<0.01) and ejection fraction (54 ± 9 versus 47 ± 7%, p=0.03) within the first 24 hours of life. These findings were not dependent on left ventricular length or mitral valve annular diameter, but were related to an increase in mitral valve inflow [mitral valve velocity time integral- (8.6 ± 1.6 versus 7.4 ± 0.9, p= 0.01)]. There was no case of PPHN diagnosed in the neonates.

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 (1). 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 0.1 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 0.1 Changes in Haemodynamics Over Time in Pregnant vs. Non-Pregnant Subjects

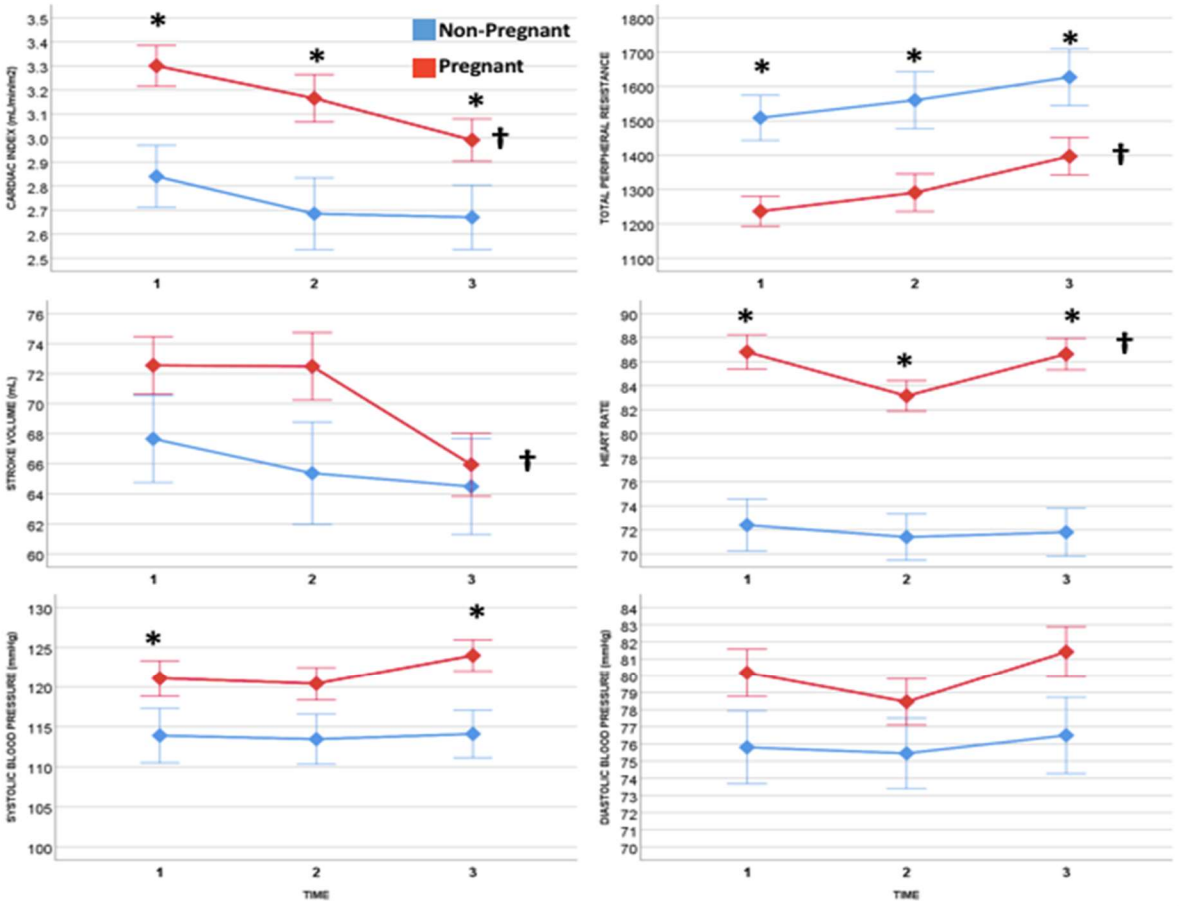


Figure1
*** = p <0.05 between groups at time point**
† = p <0.05 within group Time 3 vs. Time 1

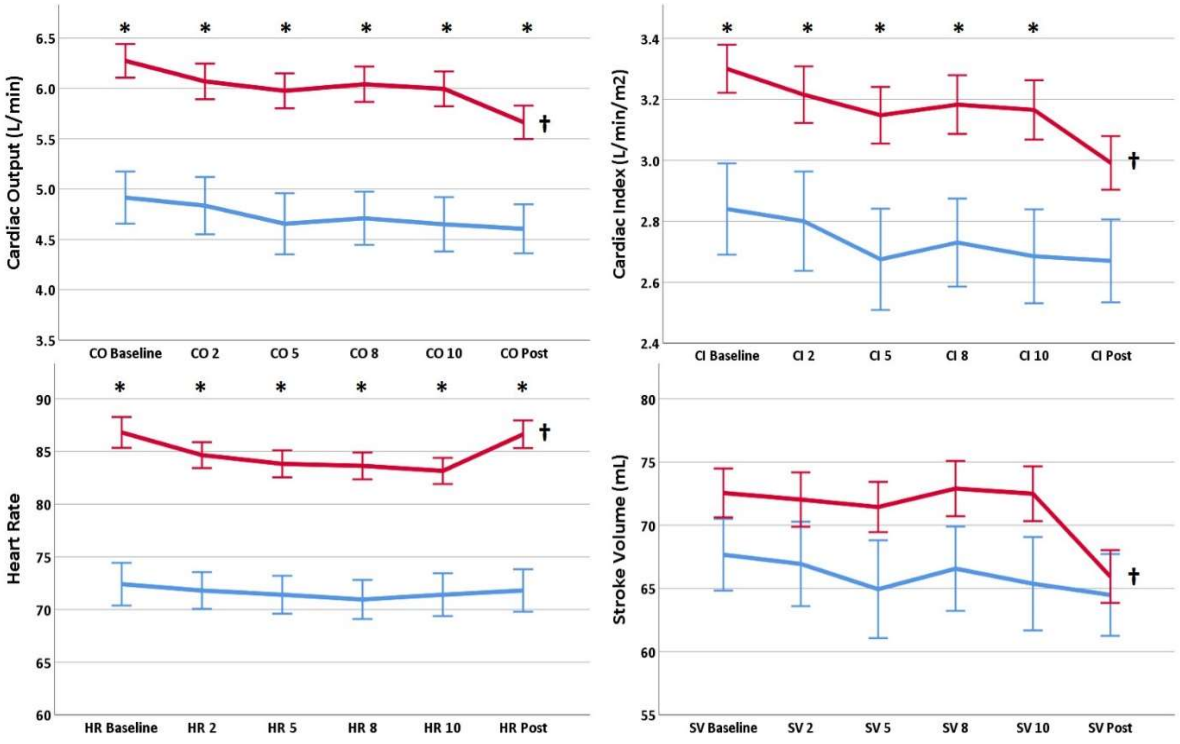
Changes in cardiac index (CI), total peripheral resistance (TPR), stoke volume (SV), heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in pregnant versus non-pregnant subjects.

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 0.2 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.

9.4 STATISTICAL METHODS PLANNED IN THE PROTOCOL & DETERMINATION OF SAMPLE SIZE

STATISTICAL AND ANALYTICAL PLANS

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).

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.

DETERMINATION OF SAMPLE SIZE

Sample sizes of between 24 (12 per group) and 50 have been recommended variously for pilot studies Following these broad recommendations and that of the NHS 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 AUT-F0316-2 47/57 [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-aFeasibility-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 2 groups (responders and non repsonders) were chosen.

The number of subjects reported to be in the baseline period are not the same as the worldwide number enrolled in the trial. It is expected that these numbers will be the same.

The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period

No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.

9.5 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

PROTOCOL AMENDMENTS

The protocol was substantially amended once during the trial (see HPRA approval for amendment in Appendix 3). The changes were as follows:

- Sample Size analysis section was revised to increase the sample size from a total of 60-75 participants to 80-95 participants. The rationale for the sample size amendment was the addition of a new cohort population (non-pregnant controls) to the study.
- For key inclusion criteria, an additional category of subjects was added: Non-pregnant controls. The rationale for this new category was to compare the haemodynamic changes in both maternal and non-maternal controls following the test product.
- The Data Safety Monitoring Board was removed from the trial protocol. The rationale for this was due to the small

number of subjects recruited to the trial and the high safety profile of the IMP administered over 10 minutes, a DSMB was not considered necessary by the Sponsor. The side effect profile of the IMP administered for a total duration of 10 minutes is negligible and at the time of the substantial amendment, no safety concerns or adverse outcomes had arisen.

10 STUDY POPULATION

10.1 DISPOSITION OF PATIENTS

Recruitment
46 pregnant patients and 20 non pregnant patients were recruited to the study

10.2 PROTOCOL DEVIATIONS

Table 5 gives details of protocol deviations observed during the study.

Table 5 Protocol deviations

Deviation #	Subject Number(s)	Description of Deviation
1	RHD 3, RHD 7, RHD 8 RHD 9, RHD 11	The protocol Version 2 included a Neonatal follow-up in the paediatric outpatient department 6 weeks after hospital discharge. Parents of babies that had no issues at discharge were reluctant to come back for the 6 week follow up and so subjects RHD 3, RHD 7, RHD 8, RHD 9 and RHD 11 did not return for their follow up visit. Subjects RHD 3 & RHD 7 were followed up by phone and the Visit 5 information was captured by phone. A non-substantial protocol amendment was put in place, Version 3, 25-Jul-2017 which included the option of a telephone visit at the 6 week post discharge follow up for those babies who did not require an outpatient follow up visit after discharge. This will apply to subjects RHD 013 onwards.
2	RHD 38, 45 & 46	For subjects 38, 45, & 46, the monitor noted on 17-Feb-2020 that machine vital signs were not taken at Visit 1 & 2 (same day for all 3 subjects). The protocol stipulates that vital signs must be taken at these visits. The sub-investigator stated that although machine vitals were not taken, the NICOM data for these 3 subjects measured their vital signs (except for temperature). The sub-investigator stated that the vital signs measured via the NICOM was sufficient for monitoring the safety of trial subjects.

11 RESULTS

11.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICs

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].

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Table 1: Demographics

Baseline characteristics (n=46)	
Mean Age (years)	31.7 (SD 7.1)
Median BMI (Kg/m²)	29.6 [IQR 27.6-33.5]
Median Gestation age (weeks)	35.0 [IQR 33-37]
Primiparous	15 (33%)
Caucasian	39 (85%)
Hb level third trimester (g/dL)	11.7 (11.1-12.7)

13 SAFETY EVALUATION

There were no Serious Adverse Events (SAE) or non-serious adverse advents during the duration of the clinical trial.

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15 APPENDICES

Appendix 1: Protocol and Protocol Amendments

Appendix 2: Ethics Committee Initial approval & approval of amendments

Appendix 3: Initial Regulatory Approval & approval of amendments

Appendix 4: Study Information