IMPROVING THE MANAGEMENT OF EXTREMELY EARLY-ONSET FETAL GROWTH RESTRICTION

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Biology, Medicine and Health

2021

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Word count: 65903

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Abbreviations

AAC	Average acceleration capacity
AC	Abdominal circumference
ADC	Average deceleration capacity
AFI	Amniotic fluid index
AGA	Appropriately grown for age
aOR	Adjusted odds ratio
AUC	Area under the curve
AUROC	Area under the receiver operating characteristic curve
BMI	Body mass index
BP	Blood pressure
BPD	Biparietal diameter
CITL	Calibration-in-the-large
CTG	Cardiotocography
cCTG	Computerised cardiotocography
CHI	Chronic histiocytic intervillositis
CI	Confidence interval
CPR	Cerebroplacental ratio
DV	Ductus venosus
ECG	Electrocardiogram
EDF	End-diastolic flow
eFGR	Extremely early-onset fetal growth restriction
EFW	Estimated fetal weight
FDIU	Fetal death *in utero*
fECG	Fetal electrocardiogram
FGR	Fetal growth restriction
FHR	Fetal heart rate
FL	Femur length
GRIT	Growth Restriction Intervention Study
HC	Head circumference
hCG	Human chorionic gonadotrophin

HDU	High dependency unit
HRV	Heart rate variability
IRR	Incidence rate ratio
IVC	Inferior vena cava
LoS	Length of stay
LR	Likelihood ratio
LTV	Long-term variation
MAP	Mean arterial pressure
MVM	Maternal vascular malperfusion
MCA	Middle cerebral artery
MMR	Mean minute range
NEC	Necrotising enterocolitis
NICE	National Institute for Health and Care Excellence
NICU	Neonatal intensive care
NND	Neonatal death
NPV	Negative predictive value
NRS	National Records of Scotland
NSS	National Services Scotland
OR	Odds ratio
PAPP-A	Pregnancy associated plasma protein-A
PEC	Placental efficiency coefficient
PIGF	Placental growth factor
PI	Pulsatility index
PIV	Pulsatility index for veins
PPV	Positive predictive value
PPROM	Preterm prelabour rupture of membranes
PRSA	Phase-rectified signal averaging
PROGRESS	PROGnosis RESearch Strategy
PSA	Placental surface area
PVIV	Peak velocity index for veins
RCOG	Royal College of Obstetricians & Gynaecologists
RCT	Randomised control trial

RI	Resistance index
ROC	Receiver operating characteristic
SDVP	Single deepest vertical pool
sFlt	Soluble fms-like tyrosine kinase-1
SGA	Small for gestational age
SMR	Scottish Morbidity Record
STRIDER	Sildenafil TheRapy in Dismal Prognosis Early-onset Fetal Growth Restriction
STV	Short-term variation
TRUFFLE	Trial of Randomized Umbilical and Fetal Flow in Europe
UtA	Uterine artery
UA	Umbilical artery
UCR	Umbilical-cerebral ratio

Abstract

Fetal growth restriction (FGR) is a common complication of pregnancy and the single largest risk factor for stillbirth in high-resource settings. FGR can be classified by the gestation at diagnosis, using 32-34 weeks' gestation to differentiate early-onset from late-onset disease. Within early-onset FGR there appears to be an extreme subset, typically diagnosed before 28 weeks' and delivered prior to 33 weeks' gestation which is associated with more severe placental disease and worse outcomes. It is this extreme subtype, termed eFGR, which forms the focus of this thesis. With no therapeutic interventions available, eFGR management is based upon antenatal surveillance using ultrasonography and fetal heart rate monitoring. The goal of management is optimise outcome, whilst avoiding preventable stillbirth. However, there is a paucity of eFGR specific knowledge about survival and outcome, and further study is required to provide better information for clinicians and parents of affected babies.

It was hypothesised that in cases of eFGR, antenatal factors, ultrasonography and FHR patterns can be used to better predict outcomes. This hypothesis was tested by: 1) Using population level and local data to determine the current incidence of eFGR and investigate the relationship between gestational age at delivery, birthweight and survival; 2) Performing a prognostic factor study to better characterise eFGR and identify if antenatal and ultrasound characteristics can be used to reliably predict outcome in individual cases of eFGR; 3) Prospectively examining the validity and potential value of ambulatory 24-hour fetal heart rate monitoring in cases of eFGR.

The incidence of eFGR was confirmed to be 3 per 1000 births in the general maternity population. The relationship between gestational age, birthweight and neonatal death in preterm infants was explored to suggest the two can be combined to predict outcome. In the case of eFGR infants with static growth, there is likely to be little advantage by gaining gestation. On an individual level, a combination of ultrasound measurements at diagnosis can be used to predict the likelihood of stillbirth. In addition, longitudinal data collected over the course of an eFGR pregnancy relating to fetal growth and Doppler progression can be used to modify risk predictions as the pregnancy progresses. Finally, a comparison of computerised cardiotocography parameters in eFGR and normal pregnancies, although highlighting differences between the two, suggests that further work is required in this area to determine how analysis of fetal heart rate can be improved as a tool for predicting prognosis in these high-risk pregnancies.

This set of studies has provided improved predictions of pregnancy outcomes for both clinicians and affected families. It has highlighted areas for further development which should translate in the future to improved management, subsequently reducing associated morbidity and mortality.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Acknowledgements

I am incredibly grateful to Tommy's for providing the funding for this project, the staff at The Maternal and Fetal Health Research Centre, and most importantly the patients who took part in the fetal heart rate monitoring study.

I would like to thank my supervisors Ed Johnstone, Alex Heazell and Emma Ingram for their support and encouragement throughout this PhD and since my journey started in this department as an MRes student 10 years ago. Ed – your enthusiasm and ability to always think of more questions has pushed me to learn and explore more than I thought could do. Alex – I will always be grateful that you believed in me enough to give me another chance in obstetrics after my brief hiatus to gynae oncology. Emma – thank you for keeping the other two in check!

I would like to acknowledge Jenny Myers for her statistical expertise, Mark Hann for his assistance with navigating the NHS Scotland application process and his statistical help with Chapter 2, and Hitesh Mistry for his suggestions of new approaches to analysis of the fetal heart rate. I'd like to thank all the other Clinical Research Fellows for the endless cups of coffee, biscuits and brainstorming / motivational chats, in particular Laura Ormesher and Alice Dempsey (as three CRFs do almost make a professor…!).

Finally, I'd like to thank my family for endless proofreading assistance, and Dave and Harriet for keeping me going and putting up with me when I wasn't sure how I was ever going to finish.

Publications arising from this work

Peer-reviewed papers:

1. Warrander LK, Ingram E, Heazell AEP, Johnstone ED. Evaluating the accuracy and precision of sonographic fetal weight estimation models in extremely early-onset fetal growth restriction. Acta Obstetrica et Gynecologica Scandinavica. 2020; 99: 364-373. doi: 10.1111/aogs.13745.

Published conference proceedings:

1. Warrander L, Ingram E, Heazell A, Johnstone E. Diurnal variation in the fetal heart rate (FHR) in extremely early-onset fetal growth restriction. BJOG. 2019; 26 (Supplement 1): 60-86, EP182. doi: 10.1111/1471-0528.15634.
2. Warrander L, Ingram E, Heazell A, Johnstone E. eFGR prevalence, prognosis and outcomes in a UK population dataset. AJOG. 2020; 222(1): S507-S508. doi: 10.1016/j.ajog.2019.11.819

CHAPTER 1: INTRODUCTION

1.1. Overview

Extremely early-onset fetal growth restriction (eFGR) is a rare, yet potentially devastating complication of pregnancy. Currently, there are no therapeutic interventions for this condition, and management relies solely on balancing the timing of delivery to minimise the risk of fetal death *in utero* (FDIU) against the risks of neonatal mortality and long-term morbidity associated with iatrogenic preterm delivery at such extremes of birthweight and gestation. There is a lack of knowledge regarding prognostic indicators, resulting in an inability of obstetricians to determine accurately the likelihood of a live birth or survival of the neonatal period on a case-by-case basis. Clinically, this means that there is no method of risk-stratifying cases, and all affected pregnancies are managed along a similar pathway, based predominantly on monitoring fetal growth and fetoplacental Doppler to detect deterioration, and assessment for the presence of co-existing maternal disease. In some cases, prognosis may be thought to be so poor that the option of conservative management, or even termination of pregnancy, may be considered. From the parents' point of view, this lack of knowledge precludes effective counselling about survival chances and long-term neonatal health, both of which are critical for an informed decision to be made about management options.

Research is required in eFGR to resolve fundamental unanswered questions surrounding the incidence of extreme early-onset growth restriction and survival statistics. Furthermore, there is a need to develop an individualised risk prediction model, which could be used to give a better indication of prognosis based on ultrasound parameters and maternal characteristics.

1.2. Fetal growth restriction

Fetal growth restriction (FGR) is a common complication in obstetrics, but is the single largest risk factor for FDIU in the developed world, conferring an eight-fold increased risk of stillbirth and accounting for almost half of all cases of FDIU prior to 34 weeks' gestation (1). Approximately 40% of all FDIU cases have a birthweight below the 10th centile (2). Despite its obvious importance, FGR is inconsistently defined, and clinical detection rates and management strategies can vary hugely, as shown by the variation in guidelines between countries and individual maternal units (3,4). Diagnosis can be based on a wide range of parameters, including estimated fetal weight (EFW) / birthweight centile, umbilical artery (UA) Doppler status, maternal uterine artery (UtA) Doppler status, cerebroplacental ratio (CPR), and potentially placental biomarkers, such as placental growth factor (PlGF). To add to the confusion, the term FGR is often used interchangeably with small for gestational age (SGA). In general, FGR is used to refer to a pathological deviation in fetal growth, whereas SGA relates to any infant born below an arbitrary birthweight centile cut off, usually less than the 10th centile. SGA, by definition, will include a mixed cohort of pathologically small and constitutionally small infants, whereas an infant can have FGR but not SGA if their weight is above the 10th centile but they have an abnormal growth trajectory. For the remainder of this thesis the term FGR will be used to refer to a pathological deviation in fetal growth.

FGR is a multifaceted condition, not easily explained by a single pathophysiology; the predominant underlying pathology pertinent to this thesis is accepted to be placental dysfunction, but other factors implicated in its pathogenesis include infectious agents (5) and genetic factors, including abnormal fetal karyotype (6) and chromosomal abnormalities (7).

FGR can be subclassified by the gestation at which it is diagnosed, with a cut off of 32-34 weeks typically used to differentiate early-onset from late-onset disease (8). Figure 1.1 summarises the main differences between early-onset and late-onset FGR. Although the rates of mortality and morbidity are much lower in late-onset FGR compared to early-onset FGR, the relatively high incidence of late-onset disease means that it still accounts for a large proportion of the adverse outcomes attributed to FGR as a whole.

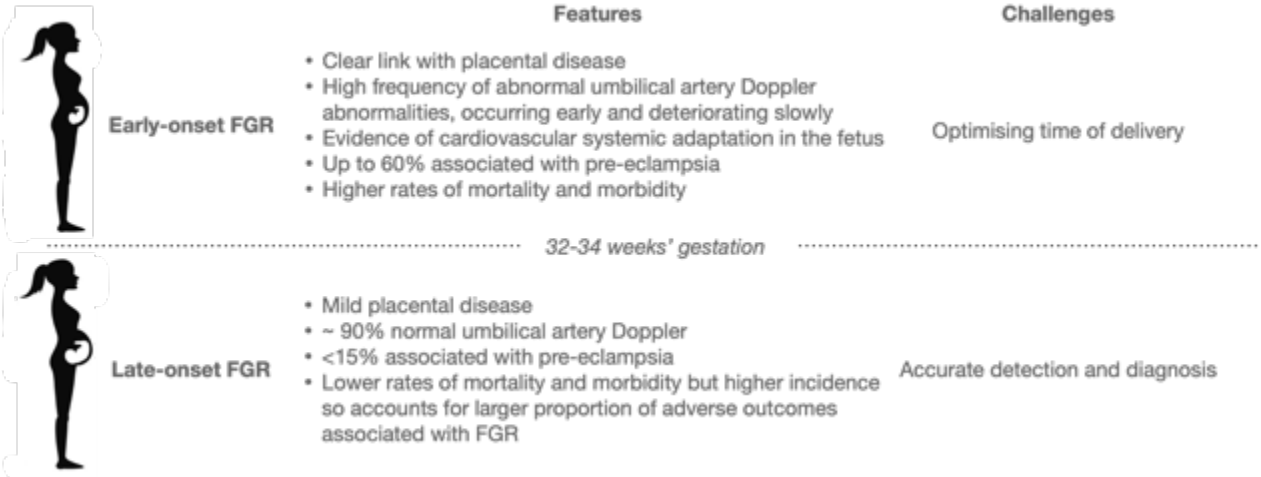


Figure 1.1: Summary of the main features and challenges associated with early-onset FGR and late-onset FGR (8–10).

1.2.1. Defining fetal growth restriction

Defining FGR is a contentious issue, but usually involves a statistical deviation of growth from a population or customised centile (typically below 3rd / 5th / 10th centile) with or without measures of placental function such as maternal or fetal Dopplers (11,12). The lack of a consistent definition of FGR prevents the accurate and consistent identification of all fetuses at risk, and from a research perspective it becomes difficult to compare results across studies. To minimise these problems, a recent international Delphi study attempted to come to a consensus definition of FGR secondary to placental dysfunction and in the absence of congenital abnormalities (8). The agreed definition is outlined in Table 1.1

Table 1.1: Consensus definition of FGR (8).

	Early-onset FGR	Late-onset FGR
Solitary factors	Abdominal circumference (AC)/EFW < 3rd centile Absent end diastolic flow (EDF) in the UA	Abdominal circumference (AC)/EFW < 3rd centile
Contributory factors	AC/EFW < 10th centile AND UtA pulsatility index (PI) > 95th centile OR UA PI > 95th centile	*2/3 of following:* AC/EFW < 10th centile AC/EFW crossing 2 quartiles on growth chart CPR < 5th centile/UA PI > 95th centile

To differentiate between early- and late-onset FGR, 32 weeks was chosen as the gestational age cut off, but no specific mention was made as to whether this referred to the gestational age at diagnosis or delivery (8).

Due to the heterogeneity of the definitions used within the literature regarding different gestational age cut offs for FGR, different subgroups of FGR will be defined as follows throughout this thesis:

> *Late-onset FGR:* FGR as per Delphi criteria, diagnosed post-32 weeks' gestation.
>
> *Early-onset FGR:* FGR as per Delphi criteria, diagnosed pre-32 weeks' gestation
>
> *Extremely early-onset FGR (eFGR):* FGR as per Delphi criteria, diagnosed pre-28 weeks, delivery by or planned for 32 weeks' gestation.

A review of the differences between early- and late-onset FGR is outside the scope of this thesis, which instead will focus on an extreme subset of early-onset FGR.

1.3. Extremely early-onset FGR

There have been two important studies over the past decade that have led us to the decision that within early-onset FGR there is a subset of extreme cases, and it this cohort that will form the focus of this thesis.

Firstly, Lees and colleagues designed the Trial of Randomized Umbilical and Fetal Flow in Europe (TRUFFLE) study, to determine which method of monitoring and triggering delivery was associated with the best neurodevelopmental outcomes in FGR diagnosed between 26 and 32 weeks' gestation (13). The findings from this study are discussed in greater depth in Section 1.4.4, but the overall outcomes in the cohort of patients recruited were generally optimistic, with an overall survival rate of 92%, a mean gestational age at delivery of 30.7 weeks and a median birthweight of 1013g (13).

Secondly, the Sildenafil TheRapy In Dismal Prognosis Early-onset Fetal Growth Restriction (STRIDER) study was an international randomised controlled trial (RCT) which aimed to determine if sildenafil could be used as a therapy to prolong gestation in cases of early-onset FGR (14). The UK study was the first to be published, and within this cohort outcomes were much less optimistic than had been seen in the TRUFFLE study, with an overall survival rate of 68%. However, comparing the cohort characteristics between the two studies reveals the STRIDER cohort to be a more extreme subset of early-onset FGR, with a median birthweight and gestation at delivery of 597g and 28.3 weeks respectively (15). This suggests that within early-onset FGR, there is a more extreme subgroup (eFGR), typically diagnosed pre-28 weeks' gestation and associated with more severe disease and worse outcomes.

With advances in modern neonatal care, mortality and morbidity is now much lower in infants born after 32 weeks' gestation (16). Infants who are diagnosed with FGR before 28 weeks often require delivery before they reach this gestation, with additional challenges in subsequent neonatal management due to their gestation and birthweight. To complicate management of eFGR further, a proportion of eFGR cases will be diagnosed in infants on the borderline of viability, often before 26 weeks' gestation. Here the first difficulty lies not in the timing of delivery, but in the choice between conservative and active management, or in some situations opting for a termination of pregnancy. Parents require careful counselling in this scenario to ensure that they have a realistic expectation of the likely outcomes and potential long-term implications (17). Unfortunately, research is so scant in this area that accurate counselling is difficult. A recent small qualitative study of six women who experienced a stillbirth or neonatal death following a diagnosis of eFGR revealed that women wanted to be informed of all possible outcomes of the pregnancy, but that sometimes the appropriate information was not able to be provided (18).

Lawin-O'Brien and colleagues reviewed outcomes in 181 pregnancies diagnosed with eFGR before 26 weeks' gestation and delivered before 32 weeks, over a 15 year period from 2000 in three fetal medicine units in the UK (17). Only 27% of infants survived, 45% of cases ended in FDIU, 10% resulted in neonatal death, and 18% of parents opted for a termination of pregnancy. Post 28 weeks', there was a dramatic change in survival, with 59% surviving and 26% ending in FDIU, compared to only 13% surviving and 53% of cases ending in FDIU if delivery was before 28 weeks' gestation (17). This is the largest study to date of outcomes and ultrasound characteristics in periviable FGR, and highlights that although mortality is almost inevitable for those delivered before 28 weeks' gestation, beyond this survival chances improve, and there are cases that progress beyond 32 weeks' to be delivered after 36 weeks' (17). Based on these results, the authors conclude that termination of pregnancy should be offered with caution, as it is difficult to predict the gestation at which an individual case may deliver, and also what the outcome of that case will be (17).

Currently, the only management for eFGR is delivery, which must be timed to balance the risks associated with delivery at such extremes of gestation and birthweight against the ongoing risk of FDIU in continuing with the pregnancy. The previously mentioned STRIDER study tested sildenafil as a therapy to prolong gestation in eFGR (15). The underlying hypothesis for this study was that sildenafil is an inhibitor of phosphodiesterase type-5, which is responsible for the breakdown of nitric oxide and can therefore enhance nitric oxide dependent vasodilatation, which has been shown to improve fetal growth in a mouse model of FGR (19). A small study of 10 cases of eFGR treated with sildenafil initially showed promise, with sildenafil treatment associated with increased fetal growth (OR 12.9; 95% confidence interval 1.3-126) (20). Unfortunately, in the larger RCT, there was no difference in gestational age at delivery, birthweight or pregnancy outcomes in those who received sildenafil compared to placebo (15).

Novel therapies are in the pipeline, most notably through the EVERREST Consortium, which plans to investigate the feasibility of using maternal adenovirus gene therapy to increase vascular

endothelial growth factor expression in the uterine arteries, with the hope that this will increase nitric oxide release (21). This has shown promise in sheep and guinea pig models (22,23), but a trial is yet to begin in humans.

There have been no prognostic studies to date in eFGR that have attempted to identify features of the disease that can be identified at presentation, or as the pregnancy progresses, and used to better predict outcomes. Having this knowledge would greatly improve the counselling offered to parents, and aid in clinical decision making surrounding the frequency of antenatal surveillance and timing of delivery. One of the main aims of this thesis will be to identify potential prognostic factors in eFGR.

1.3.1. Incidence of eFGR

Despite the realisation that eFGR is rare, there has been little attempt to date to define its incidence. eFGR is not reported separately from FGR as a whole, therefore the scope of the problem remains unknown. FGR is estimated to occur in 5-8% of pregnancies (depending on the exact definition used), and within this it has been hypothesised that 20-30% of cases are classed as early-onset, again dependent on the definition used (24). Preterm pre-eclampsia could be used as a proxy measure of the incidence of eFGR, given that the two often co-exist (25); the incidence of preterm pre-eclampsia is estimated to be around 0.3% (26).

Determining the incidence of eFGR and understanding more about its contribution to perinatal mortality and morbidity will reveal the true extent of the impact this disease has, not only from an obstetric point of view, but also from a neonatology and longer-term paediatric health point of view. Without knowing a true incidence, it is hard to correctly allocate both clinical and research resources.

1.3.2. Placental pathology in eFGR

A complete review of placental pathology associated with eFGR is outside the remit of this thesis, however a brief overview will be presented to contextualise some of the later work discussed.

Multiple different morphological placental features have been reported as associated with FGR, however there are inconsistencies amongst studies performed. Firstly, as previously discussed, the definition of FGR used varies greatly. Some studies (particularly older studies) focus more on SGA and using an arbitrary birthweight centile, or even just an actual birthweight cut off to define disease, which does not consider other features suggestive of aberrant fetal growth. This leads to a study population made up of pathologically small and constitutionally small fetuses, within which there will naturally be different histological findings, and it prevents straightforward comparison of the findings between different studies. In more recent years, using clinical biomarkers such as PlGF or maternal and fetal Doppler parameters has resulted in clearer identification of subgroups of FGR. Secondly, there can be huge variation in findings within the same placenta from an individual, therefore different sampling techniques may result in different findings even within the same case (27). Sampling protocols need to account for this potential for heterogeneity within the placenta and ensure that the placenta is adequately represented. Thirdly, the presence or absence

of certain lesions may be based on a subjective interpretation by a pathologist, who can also be subject to bias depending on the frequency with which they expect to find certain lesions, or diagnose a particular condition, or with the findings they have associated with different conditions in the past. Certainly, within the clinical environment, histopathological analysis is rarely performed without prior knowledge of the clinical condition (28). Definitions of each lesion can also vary (27). Finally, differentiating whether the presence of a particular lesion is a cause of a particular condition, or association of that condition is almost impossible in this context, and is again subject to bias based on what has previously been suggested in the literature, or seen in the pathology department. To address some of these deficiencies, an international workshop was set up to establish a uniform sampling procedure and diagnostic criteria that could be implemented across the world in both clinical and research settings to minimise the variability in histopathology reporting (27).

Despite these limitations, there are distinct patterns of pathology that are associated with different subgroups of FGR, such as early-onset and late-onset. The predominant pathological placental finding that appears to be associated with early-onset FGR is maternal vascular malperfusion (MVM), although there are a small number of other pathological findings which can be associated with early-onset disease.

1.3.2.1. Maternal vascular malperfusion

Similarities exist between lesions described in early-onset FGR and early-onset pre-eclampsia, namely the existence of MVM. This is the most prevalent histological placental finding in early-onset FGR, with one study of 196 women suggesting that findings consistent with MVM were seen in 80% of cases (29). The Amsterdam consensus describes associated macroscopic changes in MVM as placental hypoplasia (placental weight <10th centile for gestational age and/or a thin cord), infarcts and retroplacental haemorrhage (27). Associated microscopic changes include distal villous hypoplasia, accelerated villous maturation and decidual arteriopathy (27). These changes are believed to originate in the impaired transformation of the spiral arteries. Burton et al. summarised that this altered conversion results in increased velocity of blood flow into the villous space, which in turn leads to (a) physical damage of the villous tree and chorionic plate, and (b) diminished maternal-fetal nutrient/oxygen exchange, and increased intermittent perfusion, which increases the likelihood of ischaemic-reperfusion injury (30). High velocity damage to the villous tree can take the form of both macroscopic change, whereby the villous tree is forced apart, leaving intervillous lakes, and microscopic change, where fragments of trophoblast shear off under the increased hydrostatic pressure and are released into the maternal circulation, where they can cause an inflammatory response (30). Ischaemic-reperfusion injury and the subsequent increase in reactive oxygen species leads to increased formation of syncytial knots (31). Syncytial knots, which become syncytial aggregates when shed into the maternal circulation, are involved in the production of soluble fms-like tyrosine kinase-1 (sFlt-1), and continue to be transcriptionally active following their release (32,33). Exposure of the syncytiotrophoblast to oxidative stress and hypoxia (34) also results in a reduction in the secretion of PlGF, low levels of which are associated with FGR (35). The imbalance between sFlt-1 and PlGF levels in early-onset FGR are discussed further

in Section 1.3.3.1. Clinically, MVM is associated with abnormal UtA Doppler waveforms (29), with high resistance flow and/or the presence of notching, either unilaterally or bilaterally. Features associated with MVM can also be evident in apparently normal pregnancies, which highlights previously mentioned difficulties in attributing a change to a cause or an association, with one study showing it had an 8% incidence in a population of apparently low-risk nulliparous women, with only half those pregnancies having an adverse outcome (36).

1.3.2.2. Other placental pathologies associated with early-onset FGR

One single-centre study has suggested that 10% of early-onset FGR cases are associated with normal UtA Doppler waveforms, and that within this subgroup only 26% exhibited pathological changes consistent with MVM (29). Histological examination of the placenta following early-onset FGR with normal UtA flow is more likely to reveal massive perivillous fibrin deposition (37,38), chronic histiocytic intervillositis (CHI) (39), or villitis of unknown aetiology (40). These pathological findings are not attributed to changes in placental perfusion, so an association with abnormal maternal Dopplers would not typically be expected. They all, however, have a significant rate of recurrence (41), with the recurrence rate of CHI estimated to be as high as 80% (39). This is in contrast to MVM, where the recurrence rate, although still significant, is much lower at 10-25% (42).

Although understanding placental pathology in eFGR is important for counselling in the postnatal period regarding future pregnancies and can help in understanding of certain Doppler parameters that are seen, it will not be discussed further in this thesis because being a postnatal finding, it cannot be incorporated into a prognostic model.

1.3.3. Biomarkers in FGR

As an endocrine organ, the placenta produces hormones that have an effect both on the mother and the fetus, and on the placenta itself. Changes in the levels of these hormones can indicate increased risk of FGR and also in the likelihood of FDIU.

1.3.3.1. sFlt/PlGF

An imbalance in the levels of the antiangiogenic factor sFlt-1 and the proangiogenic factor PlGF is associated with pre-eclampsia as a consequence of the abnormal placentation that occurs (43). Placental expression and maternal serum levels of sFlt-1 are particularly raised in pre-eclampsia (44–46), but less so in FGR resulting in a weaker association (47). Abnormally high levels of sFlt-1 and low levels of PlGF have been shown to precede the onset of symptoms of pre-eclampsia by several weeks (43,48), with low PlGF <5th centile having a high sensitivity (96%; CI 95-98%) and high negative predictive value (98%; 95% CI 93-99.5%) for the development of pre-eclampsia within 14 days (48). Testing PlGF reduces the time to make a diagnosis of pre-eclampsia and reduces the incidence of adverse maternal outcomes (49). Using the ratio of sFlt-1 and PlGF has also shown to be of clinical benefit, with a sFlt:PlGF ratio < 38 indicative of the absence of pre-eclampsia in women with suspected symptoms (50). Low maternal serum levels of PlGF have been shown to discriminate small fetuses with placental dysfunction from constitutionally small

fetuses (35), but angiogenic markers have only really been tested in clinical trials as a diagnostic tool for pre-eclampsia.

There have been attempts to investigate the value of using the sFlt-1:PlGF ratio in FGR in conjunction with other tests such as Doppler ultrasound. One such study which looked specifically at early-onset FGR showed the likelihood of delivery within one week was very low when both the sFlt-1:PlGF ratio was < 85 (intermediate or normal) and UA end diastolic flow (EDF) was present (51). Serial measurements in early-onset FGR do not add any clinical value; however, an abnormal ratio (> 38) was seen as early as four weeks prior to delivery in cases affected by FGR and FGR with pre-eclampsia, with ratios higher in those cases where pre-eclampsia was also present (52). PlGF testing is now being adopted into NHS care as an adjunct to aid in the diagnosis of suspected pre-eclampsia, but not FGR (53). Further research is required to assess the clinical usefulness of applying this test to early-onset FGR.

1.3.3.2. Other maternal serum biomarkers

Pregnancy associated plasma protein-A (PAPP-A) is produced by the syncytiotrophoblast and acts on insulin-like growth factor binding proteins to increase the availability of insulin-like growth factors, thought to promote fetal growth (54). Low levels of PAPP-A are associated with an increased risk of FGR, as well as pre-eclampsia and preterm birth (55), potentially reflecting reduced placental mass and defective trophoblast invasion (56). First trimester serum PAPP-A below the 5th centile is associated with an increased risk of FGR and FDIU with an odds ratio (OR) of 2.9 (95% CI 1.6-5.5) and 3.6 (95% CI 1.2-11.0) respectively (57).

Human chorionic gonadotrophin (hCG) is also secreted by the syncytiotrophoblast, and promotes placental development by stimulating differentiation of cytotrophoblast to syncytiotrophoblast (58). Low first trimester levels of hCG are also associated with FGR, but the relationship does not appear to be as strong as with PAPP-A (57,59).

Alpha-fetoprotein, inhibin-A and oestriol levels have also been investigated as a tool for screening for FGR and other pregnancy complications. Several systematic reviews have concluded that although they can be used to detect pregnancies at increased risk of complications, they do not have sufficiently high sensitivity or positive predictive value, either alone or in combination, to be used as a screening tool (60–62). Reduced maternal serum levels of placental protein-13 have also been suggested as a first trimester predictive marker of FGR (birthweight below 5th centile; not specifically eFGR) (63). Despite multiple studies looking at various combinations of serum markers with other tools such as UtA Dopplers, none have been specifically investigated for their ability to predict the development of eFGR.

1.4. Survival and neonatal outcomes in preterm births

eFGR infants are by definition delivered at 32 weeks' gestation or earlier, therefore a review of the literature regarding survival in preterm births is pertinent to this thesis. Preterm birth and survival

has been extensively studied over the years, with the EPICure (64) and Epipage (65) prospective studies designed to investigate survival and longer term health in infants born preterm.

1.4.1. EPICure

EPICure investigated immediate and long-term outcomes up to 16 years of age in infants born across England in 1995 between 20^{+0} weeks and 25^{+6} weeks' gestation (64,66). This was then repeated in 2006 (EPICure2; extended to include deliveries up to 26^{+6} days) to determine how outcomes had changed over time and with changes in neonatal practice (67,68). In 1995, survival to hospital discharge for the cohort was 39%, improving from 20% for those born alive at 23 weeks' gestation to 52% for those born at 25 weeks' gestation (69). At 30 months of age, approximately half of all survivors had some form of disability (including neuromotor function, sensory or communication function, or mental and psychomotor development), with 25% being classed as severely disabled. Interestingly, there did not appear to be a relationship between disability and gestational age at delivery, but boys were more likely to be affected than girls. Eleven years later, survival had improved in this extremely preterm cohort to 66% for those born at 25 weeks' gestation (67). Disability was related to gestational age at delivery in this cohort and dropped from 45% of those born pre-24 weeks' gestation, to 25% at 25 weeks'. A similar improvement was seen in developmental scores, but rates of severe impairment remained unchanged (68). These studies provide important information regarding the effect of gestational age on survival and childhood development and disability, but do not report findings in the context of birthweight. Analysis of the median and interquartile range of birthweight for each gestational week suggests that the majority of infants were appropriately grown for gestation in EPICure2 (67), which is important within the context of providing information about preterm birth and survival for the population as a whole, but it does not offer information that can be easily translated to the eFGR population.

1.4.2. Epipage

Epipage was a similar study performed in France in 1997, and then repeated in 2011 (Epipage-2). This cohort included preterm births up to 32 weeks' gestation and had two smaller cohorts for comparison born at 33-34 weeks' and 39-40 weeks' gestation.

Survival for infants born alive before 24 weeks' gestation was less than 10%, but increased to 50% at 25 weeks' in 1997 (70), in keeping with findings from EPICure. The difference in survival before 24 weeks' could reflect different resuscitation practices between countries for periviable births, with the threshold in France for active management of preterm births being 25 weeks' gestation (71), compared to 24 weeks' in the UK. Behavioural difficulties were more frequent in very preterm compared to term infants, but interestingly, gestational age was not significantly related to the degree of deficiency (72). Epipage-2 found that survival rates for those born alive between 25 and 31 weeks' gestation improved with time, and severe morbidity was reduced, but before 25 weeks' survival continued to be rare (65), similar to the findings from EPICure. Epipage-1 considered birthweight in their analysis, and found that up to 31 weeks' gestation, infants with a birthweight $\leq 10^{th}$ centile for gestation (using centiles specifically calculated for liveborn infants of Epipage study) were more likely to die than their appropriately grown counterparts (OR 3.2; 95% CI 2.3-4.6); this analysis was not repeated in the Epipage-2 cohort (70).

1.4.3. Survival by birthweight and gestational age at delivery

EPICure and Epipage provided important survival figures by gestational age at delivery, but the birthweight is also a crucial predictor of survival. Survival figures which take into account both gestational age at delivery and birthweight have been extensively investigated by The Infant Mortality and Morbidity Studies Group at The University of Leicester (73,74). These figures were specific to infants alive at the onset of labour, and so did not give any indication of overall rates of FDIU, but they were separated according to infant sex and ethnicity. The first version was based on preterm deliveries in the Trent region of the UK during 1994-1997 (73), and was subsequently updated using data from 1998-2001. These data give some indication as to survival in SGA infants, as birthweights were included in the analysis. For example, an infant of European ethnicity born at 28 weeks with a birthweight between 1000-1249g (around the 50th centile (Hadlock (75)) had an 87% chance of survival, whereas this fell to 63% for those with a birthweight between 500-749g (less than the 5th centile) (73). This highlights the survival discrepancy between appropriately grown and pathologically small infants. However, these data were not specific to infants with a diagnosis of eFGR. eFGR status may affect the relationship between survival, gestation at delivery and birthweight to the point that survival figures based on a whole population may not be generalisable to those with eFGR.

In addition, these figures only take into consideration birthweight as a single measure. A previously unexplored method of investigating survival and prognosis is assessment of the growth trajectory prior to delivery. It could be hypothesised that those infants with static growth may have poorer outcomes following delivery than those who, although small, have continued to grow along the centile lines. This may provide additional prognostic information for counselling affected parents.

1.4.4. Survival in early-onset FGR: outcomes from the TRUFFLE study

The studies discussed previously in this section have related to survival in the population as a whole. The TRUFFLE study was the largest study to date of an early-onset FGR cohort and has provided crucial information regarding survival and neurodevelopmental outcomes in this cohort up to two years following delivery. Less than 4% of survivors had abnormal motor function at 2 years, 4% had visual impairment and 2% had hearing impairment. Two-thirds of survivors had a Bayley-II cognitive composite score of $\geq$95 (no suggestion of developmental delay). Overall, 82% survived without any impairment (76). These findings provide a relatively optimistic outlook of survival in early-onset FGR, although as mentioned previously, the cohort recruited in this study may not be representative of the eFGR cohort which is the focus of this thesis.

The conclusions from the studies described above have undoubtedly provided important and robust statistics regarding preterm birth and survival, which will influence clinical decision making and counselling, regardless of the characteristics of the population from which the data was collected. However, when considering eFGR, not only is the gestational age important in determining survival chances, but the birthweight also needs to be considered. It remains unknown which is the strongest driver of survival, birthweight or gestation, and these comparisons need to be made in order to improve counselling of affected families about the likely outcomes. In addition,

survival in eFGR infants may have a different relationship with gestation and birthweight than the relationship seen with an appropriately grown infant. Considering eFGR status in the analysis would allow this potential relationship to be uncovered. A comparison of eFGR infants with infants of a matched birthweight but gestationally less mature may help to determine the potentially beneficial effect that gestational maturation has on survival, compared to the detrimental effect of low birthweight.

1.5. Antenatal surveillance in eFGR

Given that there is no treatment for eFGR, management currently relies on intensive antenatal surveillance to determine the optimum time for delivery. Methods currently available for surveillance of high-risk pregnancies include biophysical profile, ultrasonography to monitor growth and arterial / venous Dopplers and cardiotocography (CTG).

1.5.1. Evidence from previous studies

Multiple previous studies have attempted to determine the optimum method of antenatal surveillance and delivery timing in early-onset FGR.

1.5.1.1. The Growth Restriction Intervention Trial

The Growth Restriction Intervention Trial (GRIT) was the first major study to investigate timing of delivery in FGR between 24 and 36 weeks' gestation, by comparing the effects of randomisation to immediate delivery or to delaying to the point where delivery could no longer be safely delayed (77). The hypothesis underlying the comparison was to determine if early delivery to minimise intrauterine hypoxia offset the benefits gained with prolonging gestation. The difference in randomisation to delivery interval between the two groups was four days. Overall, there was no difference in the survival to discharge rate, although there were more cases of FDIU in the delay group which was balanced by more neonatal deaths in the immediate delivery group (77). This suggests that on balance, based on the evidence available at the time of the study (conducted during 1993-2001), obstetricians were delivering at about the right time to minimise the risk of death, but this study did not investigate if there was a more appropriate trigger to time delivery. A two year follow up study of the survivors from GRIT suggested that the only difference between the immediate and delayed delivery groups was a trend towards increased disability in the immediate delivery group (78).

1.5.1.2. The TRUFFLE trial

Although the results of the TRUFFLE study have undoubtedly unified management of eFGR, further analysis of the results would raise questions regarding the finding that late ductus venosus (DV) changes are the most appropriate trigger for delivery.

The primary outcome of TRUFFLE was survival with no neurodevelopmental defects at 2 years of age. The study randomised women who were diagnosed with very preterm FGR between 26 and 32 weeks' gestation to one of three groups, which had different triggers for delivery:

1) Reduced short-term variation (STV) on computerised CTG (cCTG) (<3.5ms < 29 weeks'; <4.0 ms ≥29 weeks')

2) DV pulsatility index for veins (PIV) >95th centile for gestation (“early DV changes”)
3) Absent/reversed DV a-wave (“late DV changes”).

There were also safety-net criteria for delivery, which applied to women in all three groups:

1) Recurrent fetal heart rate (FHR) decelerations on CTG
2) Reversed EDF in the UA after 30 weeks’ gestation
3) STV < 2.6ms pre-29 weeks’; <3.0ms otherwise for those randomised to delivery based on DV changes.

The trial protocol also suggested delivery by 34 weeks’ gestation in the presence of absent UA EDF or by 32 weeks’ in the presence of reversed UA EDF, or 32 and 30 weeks’, respectively, if this was dictated by local policy (76). Having the safety-net criteria therefore indicates that although two of the three randomisation arms relied on DV Doppler changes, patients in these two arms could actually be delivered based on cCTG abnormalities or changes in the UA.

In total, 503 women were recruited to the TRUFFLE study. Of these, 317 were delivered before 32 weeks’ gestation, including 7 cases of FDIU, and this sub-group has been examined in a post-hoc analysis of fetal monitoring indications (79), the findings of which are summarised by Figure 1.2.

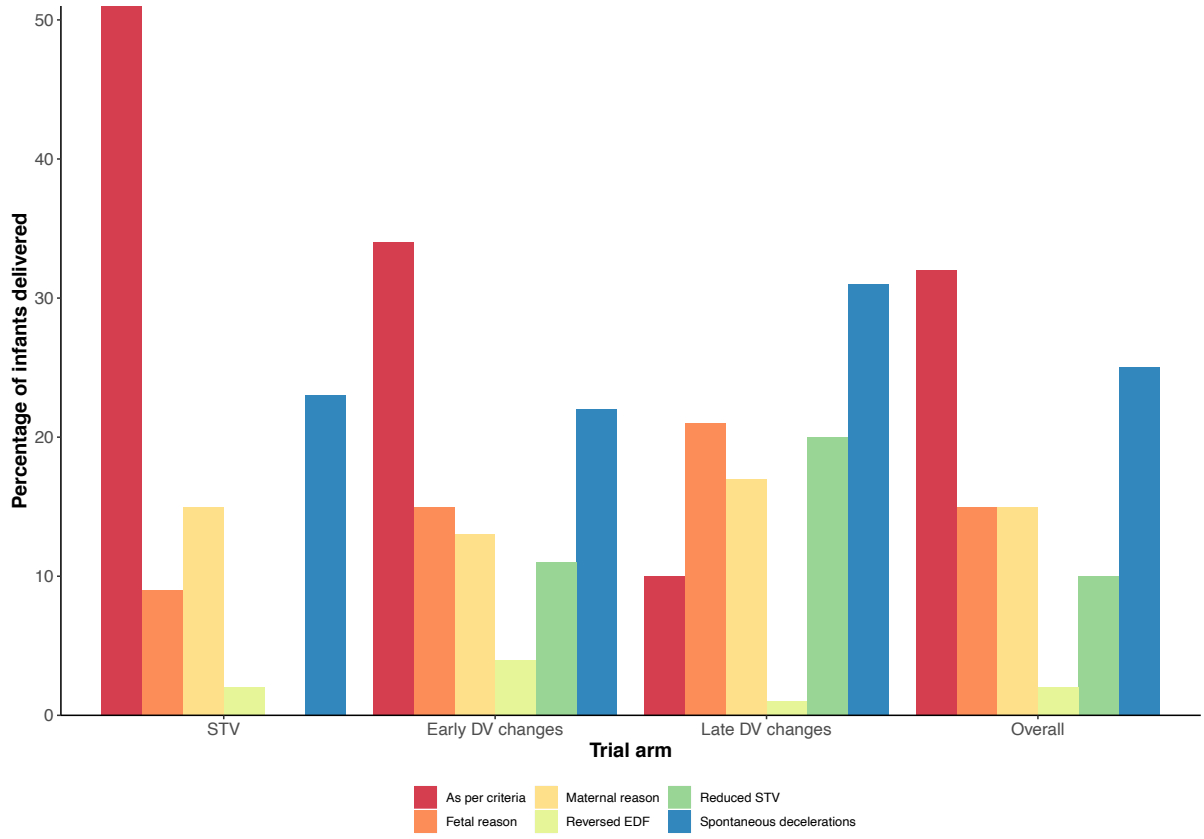


Figure 1.2: TRUFFLE post-hoc analysis to summarise the indication for delivery in each trial arm. This summarises that overall, just over 30% of infants were delivered on the basis of their allocated trial arm protocol, and only 10% of infants in the late-DV change arm were delivered on the basis of late DV changes (76,80).

From this it can be seen that within the late DV change group, which had the best neurodevelopmental outcomes, only 10% of infants were actually delivered on the basis of loss of DV a-wave. Over half were delivered due to safety-net criteria, and of those 60% were delivered due to spontaneous FHR decelerations. In the early DV change group, just over one third of infants had delivery indicated by the safety-net criteria, and again 60% of these were following spontaneous FHR decelerations on CTG. Considering all 310 infants delivered before 32 weeks, 25% of deliveries were indicated by decelerations. There is no clear definition of spontaneous FHR decelerations included within the TRUFFLE publications, therefore it is unlikely that the number of decelerations or the time period over which it occurred will be uniform across all cases. Within the late DV change group, 38% were delivered due to other fetal or maternal indications. This is higher than in the other two groups (28% in the early DV change and 24% in the low STV groups), but with no further detail included on what these indications were, it is difficult to determine if there are other criteria that should be considered when timing delivery. It is not clear from the study results if those cases had the specified abnormalities, or were delivered before these had time to occur, and this knowledge could change the interpretation of the results.

In the low STV group, the majority (51%) of infants were delivered because of the prespecified STV cut off; however, in the DV groups the majority were delivered due to the safety-net criteria, meaning that, overall, most deliveries were indicated on cCTG abnormalities. It may be that there is a sequential deterioration in the STV and DV Doppler: a reduction in STV to 3-4ms, then a deterioration in DV Doppler, and then a further reduction in STV < 3ms. This could have been further explored if information had been provided regarding the DV Doppler status of those cases randomised to Group 1 (low STV). Likewise, there are no data to indicate if those in the early DV change group showed late DV changes, or vice versa. The confusing factor is that so many infants were delivered due to decelerations, and other maternal and fetal indications, none of which have been standardised in the trial protocol. It is known that CTG interpretation can depend on the classification system used, and has a degree of inter-observer disagreement (81), so the pattern of decelerations that one obstetrician may consider to be indicative of acute fetal compromise another obstetrician may consider to be a transient self-resolving state, and it is therefore difficult to take this forward as a standard indicator for delivery. Indeed, recent work which will be presented elsewhere in this thesis has suggested that both normal and eFGR fetuses have episodes of repeated decelerations during periods of up to 22 hours of FHR recording, which are self-resolving and not indicative of an acutely compromised state (unpublished work).

One critic of the TRUFFLE study has suggested that an alternative way to perform this study would have been to have delayed randomisation to the point at which one of the three indicators tested had become abnormal (82). This would probably be difficult to implement clinically due to unwillingness on the part of either the clinicians or the participants with the risk of acute fetal compromise, but could have provided a more robust answer to the question regarding the most appropriate trigger for delivery.

The findings from the TRUFFLE study suggest a management strategy for eFGR, and this has helped to standardise management across Europe. However, a deeper inspection of the results suggests that due to the study design, the majority of the infants were not actually delivered on the basis of the pre-specified criteria. A further prospective study to evaluate this would need to be conducted in a manner that removes the potential for other delivery indicators outside of the study criteria to be a factor. Critically, the TRUFFLE study has highlighted that management of eFGR needs to combine both ultrasonography and FHR monitoring. Each of these monitoring tools will now be considered within the context of eFGR.

1.5.2. Ultrasonography

Use of ultrasound in eFGR is focussed firstly on fetal growth and estimation of fetal weight, and secondly on determination of maternal and fetal Doppler waveforms. Both of these metrics provide vital information about current fetal wellbeing, and prognosis.

1.5.2.1. Estimated fetal weight

Accurate determination of EFW is particularly crucial at the limits of viability, where it can influence the decision between active and conservative management. No studies have been performed looking specifically at the impact of EFW on survival in eFGR, but there have been studies looking at its effect at the gestational age limits of viability. In a cohort of 87 infants delivering at 23^{+0}-26^{+0} weeks' gestation, overestimation of EFW in the delivery room, immediately prior to delivery, was associated with the provision of more intensive neonatal care at birth compared to underestimation. At 6 weeks of age, survival was lower in the overestimated infants compared to the underestimated or accurately estimated infants, probably due to the fact that these infants were in fact smaller than anticipated (83). These findings are not directly translatable to the eFGR scenario, as they relate to fetal weight estimation in the delivery room setting. The majority of cases in the cohort examined in this thesis were identified prior to the decision to deliver, but this does highlight how EFW influences management decisions. Within the context of eFGR, underestimation is more likely to lead to a less active management course compared to overestimation of anticipated birthweight.

1.5.2.1.1. Accuracy of fetal weight estimation

Various factors have been investigated as exerting potential influence on the accuracy of fetal weight estimation, including acquisition and measurement of images. Fetal presentation, amniotic fluid index, gestational age at the time of scan and the EFW have all been investigated as potential factors which influence scan accuracy (84–91).

The choice of sonographic model used has been questioned as a source of error in the context of an SGA population, with multiple studies showing that the routinely used models do not perform adequately in cases of SGA. Simon et al. concluded that error was similarly increased across all models tested in SGA fetuses (92). To address these deficiencies, models specific to an SGA population have been developed, using different combinations of fetal biometric measurements (93–96), but when tested they tend to underestimate fetal weight (97). Surprisingly, when analysed as early or late SGA, using 34 weeks' as a gestational age cut off, the Hadlock BPD-HC-AC-FL (BPD: biparietal diameter; HC: head circumference; FL: femur length) model, routinely used in

clinical practice across the world, was found to be the most accurate for SGA pre-34 weeks' gestation (97). Melamed and colleagues went on to suggest that subgrouping SGA fetuses by gestational age, and applying the optimal model for each subgroup led to better estimation of fetal weight (97). Only 18% of the population studied were delivered before 34 weeks' gestation, and the mean birthweight of the whole population was 1949g, so although these findings are helpful in the context of SGA, they do not apply specifically to an eFGR population. A small study of 134 cases of preterm delivery before 28 weeks' gestation, including 51 cases of SGA, showed that EFW was more likely to be overestimated (using the Hadlock BPD-HC-AC-FL model) in SGA, with only 43% of cases estimated within 10% of actual birthweight (98). In this cohort, oligohydramnios or anhydramnios also resulted in significant overestimation of EFW in SGA compared to appropriately grown fetuses (98).

In summary, ultrasound scanning and measurement of fetal biometry is the gold standard method of estimating fetal weight, but within this there are multiple sources of error. An appreciation of potential error is key in interpreting results from ultrasound scanning and using the results in clinical practice. There is potential scope for improvement in the future, for example by incorporating new fetal biometry measurements or by developing new sonographic weight models specific to the eFGR population.

1.5.2.1.2. Growth trajectory

In addition to absolute EFW, growth trajectory is an another factor which is likely to affect prognosis. A slowing in the fetal growth rate is widely considered to be one of the defining features of FGR, yet there is no accepted definition of slowing growth, and a lack of research relating growth trajectory to pregnancy outcome. The Delphi consensus definition acknowledges that growth rate should form part of the definition, with an AC/EFW crossing 2 quartiles a contributory factor to the definition of late-onset FGR (8). Weight gain of 280g per week from 34 weeks' gestation has been linked to a reduction in the rates of operative delivery for fetal distress and admission to NICU in women with risk factors for FGR (99–101). Longer term, slowing of growth, as evidenced by the crossing of > 40 percentiles between second trimester EFW and birthweight, has been linked to accelerated growth at 2 years of age and altered cardiovascular parameters at 6 years of age, regardless of whether the final birthweight was below the 10th centile (102). This shows that fetal growth rate, or growth trajectory, is related to pregnancy outcome, suggesting that there is a critical growth rate that should be obtained to improve the likelihood of a positive outcome. Data taken from the INTERGROWTH cohort suggest that before 33 weeks' gestation, the standard weight gain in appropriately grown infants is 15g per day (83), or 105g per week; however, there has been no research to determine a minimum growth rate that could be applied to an eFGR pregnancy. Consideration of growth trajectory may represent an additional prognostic factor in cases of eFGR.

1.5.2.2. Arterial and venous Dopplers

Doppler ultrasound is crucial in the diagnosis of eFGR related to placental insufficiency, with altered parameters indicating potential altered vascular impedance and fetal compromise (103). However, they are also used to guide the frequency of antenatal surveillance and aid in the

decision for delivery. Doppler ultrasound can be applied to both maternal and fetal vessels throughout pregnancy and each contribute information about a different aspect of the pathological processes occurring in FGR. Each Doppler measurement will be considered in this section, and a brief overview of their physiology provided with the evidence as to how they can be used clinically in the diagnosis and/or monitoring of early-onset FGR.

1.5.2.2.1. Uterine artery Doppler

Abnormal UtA Doppler flow indicates an increased likelihood of placental insufficiency. It plays an important role in screening for FGR, and is now recommended in most national guidelines, including the Royal College of Obstetricians and Gynaecologists (RCOG) Green Top Guidance (104) and the latest Saving Babies Lives care bundle (105).

1.5.2.3.1.1. Physiology of uterine artery Doppler changes

In the non-pregnant woman, resistance is high in the UtA vessels, with a rapid rise and fall in the systolic velocity, and a diastolic notch. During pregnancy, endovascular trophoblast cells invade the decidua and myometrium and migrate along the maternal spiral arteries, resulting in remodelling and loss of the smooth muscle. This remodelling creates a low-resistance, high-flow state in the intervillous space to maximise the exchange of nutrients and oxygen and the removal of waste products between the maternal and fetal blood supplies (106,107). This state is reflected in a change in the UtA Doppler parameters, with a higher diastolic velocity and loss of the diastolic notch, and a gradual change from a high to low resistance state seen as gestation increases (108). As previously discussed, the pattern of MVM that is commonly seen in early-onset FGR is associated with a reduction in the usual trophoblast invasion and remodelling of the spiral arteries (107,109). This results in a continued high impedance to blood flow, manifesting as raised PI or RI and/or the presence of diastolic notching in the shape of the UtA waveform.

1.5.2.3.1.2. Uterine artery Doppler screening in FGR

Abnormal UtA resistance is associated with an increased risk of FGR and pre-eclampsia, with a normal resistance conferring a low risk of subsequent development of placental pathology (110). However, there has been much debate in the literature regarding the best time to assess UtA Doppler flow. A systematic review to assess the accuracy of UtA Doppler performed at 11-14 weeks' gestation to predict FGR and pre-eclampsia found that across 18 trials (and 55,974 women), the sensitivity and specificity for the prediction of early-onset (gestational age cut off not specified) FGR was 39.2% and 93.1% respectively, and for pre-eclampsia was 47.8% and 92.1% respectively in low-risk populations (111).

In the second trimester, UtA Doppler measurements across multiple studies suggest that the positive likelihood ratio (LR+) for FGR at any gestation following detection of abnormal UtA flow was 3.67 (95% CI: 3.33-4.03), whereas for a normal result it was 0.80 (95% CI: 0.78-0.82) (110). The degree of abnormal flow also appeared to be related to the severity of FGR and the likelihood of early delivery (110). Looking specifically at the largest study by Papageorghiou and colleagues, who assessed UtA Doppler flow in 7851 women at 22-24 weeks' gestation, the sensitivity of UtA PI > 95th centile (for that population) for the prediction for pre-eclampsia with FGR was 69%, and for

FGR without pre-eclampsia was 13% (112). In those requiring delivery before 32 weeks', this increased to 93% and 56% respectively, suggesting that screening for abnormal UtA Doppler flow at 23 weeks' gestation identifies the majority of women who subsequently have an adverse pregnancy outcome (112). It is important to note that this study assessed UtA Dopplers performed transvaginally. However, a similar smaller study which assessed transabdominal UtA Doppler found similar results (113), suggesting this technique is equally effective as a screening tool at 23 weeks' gestation. UtA Dopplers have no screening ability in the third trimester, but they may provide evidence for a suspected placental cause for FGR (114).

1.5.2.3.1.3. Longitudinal change in uterine artery Dopplers

There has been relatively little research looking at how the temporal change in UtA Doppler flow throughout gestation is related to placental dysfunction. In preterm pre-eclampsia, UtA PI is reported as significantly increased from the first trimester of pregnancy compared to normal pregnancies or pregnancies affected by term pre-eclampsia or gestational hypertension, with a smaller than expected reduction in PI across gestation (115). This fits with the pattern of impaired trophoblast invasion that occurs in preterm pre-eclampsia. The relationship between temporal UtA PI change and pregnancy outcome (for example, live birth compared with stillbirth) was not, however, explored in this study, and it may be that the rate of change could be used to derive additional prognostic information. No demographic data were presented regarding birthweight or fetal size in this study, and to date this relationship has not been explored in a cohort of FGR-affected pregnancies; it would be expected that a similar pattern would be seen in eFGR but further work is required to confirm or refute this.

1.5.2.3.2. Umbilical artery Doppler

Use of the UA Doppler as a monitoring tool in high-risk pregnancies has been linked to a reduction in obstetric intervention and perinatal deaths (116), and the RCOG advocates its use as the primary surveillance tool in SGA fetuses (104). It has both a diagnostic and prognostic use in the context of early-onset FGR.

1.5.2.3.2.1. Physiology of umbilical artery Doppler changes

In a normally functioning placenta, the waveform captured by UA Doppler shows forward flow throughout the whole fetal cardiac cycle. As gestation advances, the resistance in the UA is expected to decrease as the number of tertiary stem villi (and thus the size of the vascular bed) increases, while EDF is maintained. Abnormalities in the placental circulation that increase resistance, such as obliteration of the villous vascular tree, result in progressively reduced EDF, which is reflected in a progressively increasing PI, until a critical point where the EDF becomes absent, and then reversed. Indeed, it is believed that abnormal UA Doppler parameters are not seen until 60% of the small muscle arteries in the placenta are obliterated (117). There does not appear to be a clear progression of placental lesions when examining placentas from pregnancies affected by absent and reversed EDF, but absent EDF cases show more occlusive lesions, and reversed EDF cases show more features consistent with remodelling (118).

1.5.2.3.2.2. Umbilical artery in antenatal surveillance

A recent Cochrane review of 18 studies involving over 10,000 women concluded that the UA Doppler is recommended as a tool for antenatal surveillance in pregnancies at high risk of placental insufficiency across all gestations. However, the frequency of surveillance and timing of delivery following a finding of an abnormal Doppler measurement is not clear (116). The PORTO study found that 86% of adverse outcomes in FGR were associated with an abnormal UA Doppler, which was by far the most common Doppler abnormality (119).

Absent and reversed EDF in FGR is associated in the short-term with an increased incidence of NICU admission, and in the long-term with increased incidence of neurological damage (120). The physiological progression in UA flow as the disease progresses, is reflected in a predictable deterioration clinically (121). “Early” changes in the UA (increasing PI, absent EDF) can be present for days to weeks prior to delivery (121,122), but the deterioration to reversed flow can be gradual (122). Reversed EDF is an end-stage abnormality with a significant association with perinatal morbidity and mortality of 50% (123), appearing 4-5 days before delivery (121). The interval between abnormal UA flow and delivery is also influenced by a number of factors, including gestational age, and the presence of maternal hypertension which may necessitate delivery prior to further deterioration in the UA (122).

1.5.2.3.3. Middle cerebral artery

There is much debate over the role of middle cerebral artery (MCA) Doppler monitoring in eFGR, with insufficient evidence from prospective studies to support its use as a routine screening or monitoring tool at present. The upcoming TRUFFLE-2 study aims to determine whether randomising infants to immediate or delayed delivery on the basis of an abnormal umbilical-cerebral ratio (UCR) affects short- and long-term outcomes. TRUFFLE-2 will focus on FGR after 32 weeks’ gestation, so the findings will not be directly translatable to eFGR (124).

1.5.2.3.3.1. Physiology of middle cerebral artery changes

In a normal healthy fetus, the MCA demonstrates a high resistance flow. In the presence of hypoxia, as is presumed in FGR, blood flow undergoes redistribution to prioritise cerebral blood flow over perfusion of other less essential structures. This is achieved by a reduction in the resistance in the MCA: the so called “brain-sparing effect” (125).

1.5.2.3.3.2. Use of middle cerebral artery Doppler in surveillance

Different metrics can be taken from the MCA Doppler. Firstly, the peak systolic velocity can be used (a) as an indicator that the correct vessel is being sampled, and (b) for screening in fetal anaemia, and therefore will not be discussed further in the context of eFGR. Secondly, the PI can be used as an independent value, and compared to known reference ranges, or it can be compared to the UA PI, to give either the CPR (MCA:UA ratio), or the umbilical-cerebral ratio (UCR) (UA:MCA ratio). Using a ratio is believed to be a more sensitive indicator of compromise, as it takes into account both placental dysfunction and the fetal response (126). Both ratios are comparable, but the UCR diverges to infinity, whereas the CPR will tend to zero. Several studies have established that there seems to be a relationship between a low CPR, signifying blood flow

redistribution, and an increased likelihood of emergency operative delivery, neonatal intensive care unit (NICU) admission and metabolic acidaemia, regardless of FGR status, but these studies focussed on a population of term or near-term infants (127–129). Fetal CPR has been proposed as a method of detecting placental dysfunction in appropriately grown infants, and therefore is potentially a better indicator of late-onset FGR (130). A meta-analysis of studies comparing the prognostic accuracy of CPR and MCA Doppler to UA Doppler for adverse perinatal outcome has suggested there are few studies of sufficiently high quality to suggest it provides added value (131). CPR does predict the need for emergency delivery better than UA Doppler alone at later gestations, but for the other adverse outcomes investigated, prognosis accuracy was no better than for UA Doppler (131).

1.5.2.3.4. Ductus venosus

Ductus venosus assessment has been in use in fetal medicine for over 25 years, and is key in antenatal monitoring of fetuses at risk of cardiovascular compromise, such as those affected by eFGR (132).

1.5.2.3.4.1. Physiology of ductus venosus changes

The ductus venosus (DV) is a branch of the umbilical vein, and shunts oxygen-rich blood away from the fetal liver and directly into the inferior vena cava (IVC) to supply the coronary and cerebral vessels (133). This jet of blood joins the IVC immediately inferior to the heart, and has the highest velocity of any blood entering the heart, which allows it to preferentially open the foramen ovale and pass directly into the left atrium, allowing oxygen rich blood to rapidly enter the left ventricle and supply the coronary and cerebral circulations (133). Approximately 30% of umbilical blood is shunted through the DV in early pregnancy, but this falls to approximately 20% by 30 weeks' gestation (134). The degree of shunting also appears to be related to hypoxia, with the fetus appearing to adapt to hypoxia by increasing the volume of blood shunted through the DV, presumably to maintain adequate oxygenation of the fetal heart and brain (133). Severely growth restricted (and therefore chronically hypoxic) fetuses have shown a greater diversion of blood through the DV (135).

1.5.2.3.4.2. Use of ductus venosus Doppler in surveillance

In eFGR, DV monitoring is an integral part of antenatal surveillance (76,136,137), with loss or reversal of the a-wave representing late cardiovascular changes in the fetal circulation. Increasing placental resistance causing an increased afterload, and myocardial hypoxia causing a reduction in cardiac compliance can both contribute to a reduction in forward venous flow in the a-wave and indicate a state of fetal decompensation (132).

Loss or reversal of the a-wave indicates an increased chance of FDIU, with the risk doubling for each continued day of gestation *in utero*, and death is likely to occur within seven days if delivery does not occur (138). The TRUFFLE study was the first prospective study to investigate DV changes as an indicator for delivery, and concluded that using loss or reversal of the a-wave, when used with cCTG safety-net criteria to indicate delivery in FGR diagnosed before 32 weeks'

gestation resulted in better neurodevelopmental outcomes at 2 years of age (76). Increasing DV PIV is associated with worsening acidaemia at birth (139).

1.5.2.3.5. Temporal change in Doppler parameters

None of the Dopplers described here should be considered in isolation in the management of eFGR. There is a semi-predictable sequence of Doppler deterioration in early-onset FGR. A study focussing on early-onset FGR, albeit in a small cohort (n = 26), confirmed that early changes are seen in the UA (rising PI, then absent EDF) and MCA Dopplers (brain sparing), whereas late changes generally involve reversed EDF in the UA and absent/reversed a-wave in the DV and are significantly associated with perinatal death. Early changes were seen in 50% of patients 15-16 days prior to delivery, whereas the late changes appeared 4-5 days prior to delivery (121). A larger prospective study involving 104 cases of early-onset FGR highlighted that by the point of delivery (median 33.4 weeks) almost all cases had abnormal UA flow, half had evidence of brain sparing and one-third had an abnormal DV Doppler (140). Doppler abnormalities that presented earlier deteriorated more rapidly than those that presented later, which tended to progress slower. The authors proposed that there were three distinct patterns of Doppler deterioration: firstly, abnormalities confined to the umbilical/cerebral vessels, which progressed slowly; secondly, abnormalities progressing to the DV, but not presenting until 29 weeks' gestation; thirdly, abnormalities involving the DV, but presenting earlier (26 weeks') and progressing faster (140). Between the three groups, the only significant difference in outcome was that infants in the early presentation group were more likely to be delivered on the basis of fetal indication than those in the later onset umbilical/cerebral group.

The PORTO study questioned this typical sequence, concluding that although the expected sequence of deterioration in the UA, followed by the MCA, then the DV does exist in some fetuses, there is no single Doppler deterioration sequence that appears to prevail (119). The PORTO study also suggested that UA and MCA abnormalities are stronger predictors of adverse outcome than DV abnormalities (119). Although this study focussed on FGR (EFW < 10th centile), the mean gestational age at delivery was 37.8 weeks, suggesting that the study mainly focussed on late-onset FGR, therefore these findings may not be replicated in the context of eFGR or early-onset FGR only.

Although the evidence does not uniformly support a predictable change in Doppler waveforms, it is widely accepted that worsening flow in the umbilical and venous systems is an indicator of fetal compromise (103). Therefore, monitoring of fetoplacental Doppler is crucial to antenatal surveillance and delivery timing in early-onset FGR.

1.5.3. Antenatal fetal heart rate monitoring

Since the latter half of the twentieth century, antenatal and intrapartum monitoring of fetal wellbeing in high risk patients after 26-28 weeks' gestation has been performed using a Doppler ultrasound transducer to monitor the FHR and create a CTG (141). A "normal" CTG is considered reassuring and representative of an uncompromised fetus. However, an "abnormal" CTG is not always related to fetal hypoxia or metabolic acidosis (141). Therefore, although as a screening tool CTG has a

high sensitivity and negative predictive value, it is lacking in specificity and positive predictive value (142). The National Institute for Health and Care Excellence (NICE) has published guidance to aid and unify interpretation of intrapartum FHR patterns (143). Despite this, CTG interpretation is still subject to a varying degree of both inter- and intra-observer variation and can therefore result in false reassurance, or conversely, inappropriate intervention (144). To overcome this subjectivity in the antenatal period, the Dawes-Redman computerised analysis was developed (145). This is based on over 100,000 historically recorded antenatal CTG traces with known pregnancy outcomes and uses numeric criteria to objectively assess the trace for normal parameters. This method removes intra-observer variability and subjectivity, and analyses parameters, such as the STV, that cannot be assessed visually. Computerised CTG (cCTG) is believed to be related to improved fetal outcomes, unlike conventional visual interpretation of the CTG, which has been shown to have no positive effect on neonatal morbidity when used antenatally (146).

1.5.3.1. Computerised CTG

The need for an automated method of CTG analysis to remove the variation in visual interpretation was recognised as early as the 1970s. In response to this, a computerised analysis system was developed over 15 years in the high-risk pregnancy unit in Oxford, UK, and marketed commercially in 1991. Since 1983, over 2500 antenatal FHR traces have been archived annually, resulting in a database which currently holds over 100,000 antepartum CTGs, all of which contribute to the resulting analysis algorithm (147). The algorithm relies on several aspects of the CTG including the baseline FHR, accelerations, decelerations, short- and long-term variation, fetal movements and fast sinusoidal patterns, and compares these parameters to known normal limits according to the gestational age of the fetus (147). If the cCTG is deemed to be normal, the computer advises that monitoring can be stopped, which can be as soon as 10 minutes. If the cCTG is not normal after 60 minutes of recording, the computer highlights the reason for the abnormality to direct further investigation if necessary. This offers several advantages over conventional CTG analysis: the subjectivity of visual analysis is removed and cCTG monitoring can be reduced to as little as 10 minutes in some cases.

1.5.3.2. Dawes-Redman criteria

The analysis is initially performed after 10 minutes of recording and is subsequently updated every 2 minutes. Firstly, each minute of recording is split into 16 3.75 second epochs, over which the average FHR is calculated both as beats per minute, and as a pulse interval. For example, a FHR of 120 beats/minute has a pulse interval of 500 milliseconds.

1.5.3.2.1. Baseline

Baseline FHR is established prior to the identification of accelerations / decelerations. A filter is applied to the FHR to remove any readings outside predefined upper or lower limits. These limits are set using the frequency distribution of the epoch-by-epoch average pulse intervals to identify a peak value which represents the pulse interval, and then setting the upper and lower limits 60 milliseconds above and below this. For example, a peak of 440 milliseconds corresponds to a filter of 380-500 milliseconds, or 120-160 beats per minute (148).

1.5.3.2.2. Accelerations and decelerations

An acceleration is defined as an increase in the FHR above the baseline of more than 10 beats per minute and lasting longer than 15 seconds. A deceleration is defined as a decrease in the FHR below the baseline of either at least 20 beats per minute and lasting for at least 30 seconds, or at least 10 beats per minute and lasting for at least 60 seconds. Decelerations can be measured in 'lost beats', which is the difference between the actual number of beats and the expected number of beats had the deceleration not occurred (149). For example, if the FHR drops from 150 beats per minute to 120 beats per minute for 3 minutes, then returns to 150 beats per minute, there will have been 360 beats (3 x 120). If the FHR had not dropped there would have been 450 beats (3 x 150). The deceleration has a lost beats area of 90 beats (450 – 360).

1.5.3.2.3. High and low variation

Episodes of high and low FHR variation are only taken from periods of the recording that do not contain a deceleration or have less than 50% signal loss. For each minute, the maximum FHR above and below the baseline rate are determined and summed to give the minute range. The minute range for consecutive minutes is averaged to give the mean minute range (MMR), or long-term variation (LTV). If the minute range in least 5 out of 6 consecutive minutes is less than a pulse interval of 30ms, then an episode of low variation is identified. If the minute range in at least 5 out of 6 consecutive minutes corresponds to a pulse interval of greater than 32ms then that episode is defined provisionally as high variation, and if the MMR for that entire episode is above the 1st centile for that gestation then the episode is definitively defined as a high variation episode (150). These episodes of high and low variation are thought to correspond to the fetus cycling between periods of active and quiet sleep, with active sleep being associated with high variation and accelerations, and quiet sleep associated with low variation. Active sleep is taken to represent fetal wellbeing, but an episode of low variation during quiet sleep cannot be distinguished from the low variation associated with fetal compromise (151). For this reason, fetal wellbeing is not assessed during periods of low variation, and a trace needs to have at least one episode of high variation to be deemed normal. Healthy fetuses can have periods of quiet sleep lasting up to 50 minutes, which is why the CTG must be continued for at least 60 minutes before criteria can be deemed as not met (150).

1.5.3.2.4. Short term variation

STV is traditionally measured using the beat-to-beat variability from the fECG, however conventional CTG monitoring relies on Doppler ultrasound monitoring, which cannot measure the beat-to-beat variability. A modified STV which relies on epoch-to-epoch variation has therefore been developed. Again, this is calculated only from portions of the recording which do not contain any part of a deceleration or have less than 50% signal loss. Firstly, the difference between the average pulse intervals of adjacent 3.75 second epochs is calculated. The differences are then averaged over each minute, and then the one-minute averages are averaged over the whole trace. The two-step method of averaging the pulse interval differences prevents bias towards episodes of low variation, as episodes of high variation are more likely to be associated with a greater signal loss (152). In a trace lacking high variation, the STV correlates with metabolic acidaemia and an increased risk of FDIU, as will be discussed further in Section 1.5.3.3. (153).

1.5.3.2.5. Basal heart rate

This is the average FHR throughout all episodes of low variation, or is identified using the frequency distribution of the pulse interval as described in the baseline FHR (152).

1.5.3.2.6. Normal criteria

There are multiple normality criteria against which the recording is assessed. These are summarised in Table 1.2. If normality is not met, the reason for this will be displayed on the trace, and further action is to be taken by the clinical team.

Table 1.2: Dawes-Redman criteria for normality (152).

1	One episode or more of high variation
2	STV > 3.0ms OR if <4.5ms averaged LTV across all episodes of high variation >3rd centile for gestational age
3	No evidence of sinusoidal rhythm
4	At least one acceleration or more than 20 fetal movements per hour and an average LTV across all episodes of high variation >10th centile for gestational age
5	At least one fetal movement or three accelerations
6	No decelerations >20 lost beats if recording >30 minutes, or no more than one deceleration of 21-100 lost beats if recording >30 minutes and no decelerations >100 lost beats
7	Basal heart rate between 116-160 beats per minute if recording >30 minutes
8	LTV within 3 standard deviations of its estimated value for gestation or STV >5.0ms OR an episode of high variation with >0.5 fetal movements per minute OR basal heart rate $\geq$120 beats per minute AND signal loss <30%
9	Deceleration or artefact at the end of the recording if the recording is <60 minutes, if so, the recording should be continued.

1.5.3.3. STV and relationship with acidaemia

Repeated decelerations in an antenatal CTG have historically been associated with fetal distress in labour and an increased likelihood of birthweight below the 10th centile (154), although the exact definition of repeated was unclear in this work. The presence of repeated decelerations was key, however, as this was thought to be a better predictor of a poor outcome than a trace displaying reduced variability and an occasional deceleration antenatally (154). Following the advances in computerised analysis and the development of an algorithm to calculate LTV, it was noted that this was a more accurate indicator of fetal compromise and chronic hypoxaemia (153), and also FGR without acidaemia (151). However, using this metric failed to identify a case of FDIU where a slow sinusoidal rhythm on an otherwise steady basal FHR gave a falsely reassuringly high LTV (153). This led to the development of a further algorithm to calculate the STV, which was hoped to be a better discriminator of fetal compromise. This algorithm was tested on 7396 high-risk antenatal cCTG traces recorded between 1983 and 1987. An STV cut off of <2.5ms identified all cases that ended in FDIU, and 75% (n=4) of cases that were preterminal with metabolic acidaemia at birth (153). The association between STV within 24 hours of delivery and pregnancy outcome in 257 cases of FGR (birthweight <3rd centile) was investigated to assess the clinical value of the STV in timing of delivery in these cases. The risk of metabolic acidaemia was found to increase with a decrease in STV, and a sharp increase was seen in the likelihood ratio when the STV dropped below 3ms (155). Combining additional cCTG parameters, such as decelerations, did not improve the predictive value, and a low STV was shown to predict the metabolic acidosis with 81.3% accuracy. Fetuses delivered with an STV $\leq$3ms were significantly smaller and delivered at earlier

gestations than those >3ms, and the majority of the neonatal deaths in the study occurred in this group (155). The authors concluded that a cut off of 3ms was the best indicator for delivery timing, particularly within the context of preterm fetuses, although they acknowledge that it should be used in combination with other monitoring tools, such as DV Doppler (155), a finding which has subsequently been confirmed by the TRUFFLE study (76).

1.5.3.4. Longitudinal monitoring

In a study of 60 fetuses with FGR (AC <5th centile for gestational age) delivered pre-32 weeks' gestation, STV began to decrease from 21 days prior to delivery, and differed from the expected for gestation by 2 standard deviations from 7 days prior to delivery (156). This change mirrored an increase in DV PIV and was thought to represent a more acute fetal compromise than changes in the UA (156). It is hypothesised that this fall in STV reflects a reduction in the activity of the autonomic nervous system, in particular reduced vagal modulation (157), potentially coinciding with the onset of cardiac failure in the fetus (156). A secondary analysis of sequential FHR recordings obtained in the TRUFFLE cohort revealed further information regarding longitudinal STV changes. The results confirmed a slow decline in STV over the last 3 weeks immediately prior to delivery, with the biggest drop in the last 24 hours before delivery (158). There was however no association between longitudinal change and pregnancy outcome. Wolf *et al.* were able to show using the TRUFFLE data that the magnitude of change in STV over the 3 weeks preceding delivery in the TRUFFLE cohort was much smaller (0.8ms reduction over 3 weeks) than with the other cohort described above (approximately 4ms reduction over 3 weeks, assuming a similar variation in STV) (158). In addition, they suggested that the chance of having an abnormal STV on any particular day was 5%, but there was no way of predicting this based on previous STV readings, and the authors therefore recommended at least daily cCTG monitoring (158).

A small study (of 13 fetuses, with a range of gestations) has investigated the change in LTV in FGR, and shown a similar pattern, suggesting that a fall in LTV is also seen approximately 3 weeks prior to delivery, and at a similar time to the appearance of repeated decelerations (159).

Importantly, cCTG removes the subjectivity from CTG analysis, and in terms of antenatal surveillance, provides a reliable method of checking fetal wellbeing. In particular, STV is shown to be predictive of fetal compromise and therefore has a role in monitoring in eFGR, although the data discussed here suggest that it is of most use in the period immediately prior to decompensation. The predictive value of other parameters, such as decelerations, remains unclear.

1.5.3.5. Fetal electrocardiogram as an alternative to Doppler

The majority of FHR monitoring is performed using a Doppler ultrasound transducer, but transabdominal fetal electrocardiogram (fECG) monitoring is also an option. Technical issues have meant that this technique has lagged behind conventional Doppler ultrasound monitoring of the fetal heart rate. Given the small size and low voltage emission of the fetal heart compared to the adult heart, there is a low signal-to-noise ratio. Other factors contributing to this low ratio include electrical activity from maternal muscle activity and amniotic fluid and maternal tissues between the fetus and the abdominal surface, which contribute to non-homogeneous tissue conduction (160).

The FHR is much faster than the adult heart rate, the electrical signal received from each electrode can change regularly due to fetal movements *in utero*, and the shape of the waveform received can vary depending on the fetal presentation, posing further difficulties to overcome (160). Despite these challenges, several fECG monitoring systems have been developed, including the Monica AN24 (Figure 1.3(a)). This records the fECG, maternal ECG and uterine muscular activity through five adhesive ECG electrodes on the maternal abdomen, connected to a battery-charged portable

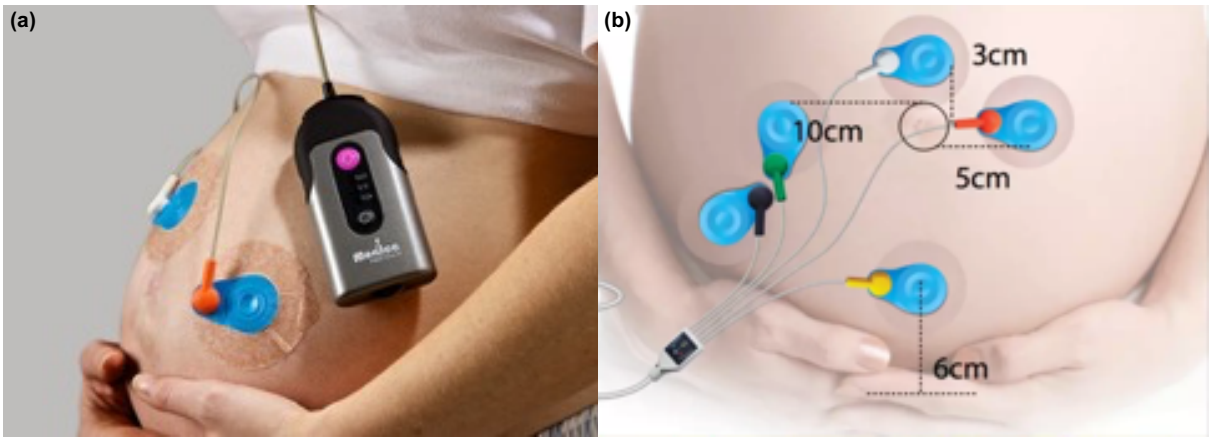


Figure 1.3 (a): The Monica AN24 device; **(b)** Electrode placement for fECG monitoring.
Source: http://www.monicahealthcare.com/. Last accessed 13th Jan 2018.

recording device (Figure 1.3(b)). As this eliminates the need for transducers and belts, it allows the wearer to remain mobile throughout the recording period, with the device able to record up to 24 hours of continuous FHR trace.

Several studies have evaluated the use of fECG compared to conventional CTG in antenatal low-risk women (161–163), and a single study has reported on the use of fECG to describe FHR patterns at 20-24 weeks' gestation (164). Evaluating signal quality at a range of gestations and maternal BMI values, Sanger and colleagues showed that pre-26 weeks', fECG has a superior signal quality compared with conventional Doppler CTG (75.5% compared to 45.3%; P = 0.003), but between 27-36 weeks' gestation, Doppler CTG offered a better signal quality (83.0% compared to 72.3% with fECG; P = 0.001), presumably due to the negative conduction effect of the vernix caseosa, which has been confirmed in other studies (165). There was no difference in signal quality at term. Increasing BMI was shown to negatively influence the signal quality of Doppler CTG, but it did not have an effect on recording of the fECG (162). Graatsma and colleagues also confirmed that BMI did not significantly influence signal recording quality between 20-40 weeks' gestation (166). Graatsma and colleagues have investigated the feasibility of long-term recordings, up to 15 hours in length. They showed that over eight hours of consistently high quality (<40% signal loss) recording could be obtained, and that the signal quality was even better during portions of recordings that took place overnight (161). This suggests that fECG is a viable alternative to Doppler FHR recording.

The ability of fECG monitors to capture the FHR at extremes of gestation has allowed further information to be gained regarding second trimester normal FHR patterns, which, with advances in neonatal care pushing the limits of viability, provide vital information for clinicians managing infants at such extremes of gestation. The basal HR is slightly higher at earlier gestations, which is thought to be reflective of a higher sympathetic drive earlier in pregnancy, which is overridden by the

increase in parasympathetic drive as the fetus matures. Overall, there were more accelerations and decelerations than are generally seen at later gestations. The mean STV in this cohort was 6.2ms, suggesting that an STV < 3ms even in this cohort is likely due to a pathological process, rather than immaturity of gestation (164).

Measurement of the fECG allows more precise detection of fetal beat-to-beat characteristics, which allows fetal heart rate variability (HRV) to be analysed. Analysis of the fetal HRV is a relatively new field, but there has been interest in the adult HRV for a number of years. HRV refers to the physiological variation in the time interval in between successive heart beats and reflects autonomic nervous system regulation (157). Both elevated and reduced HRV in the adult are related to an increased risk of mortality associated with cardiac conduction abnormalities and myocardial infarction respectively (167), therefore it seems reasonable to assume that measures of HRV could also be used to identify pathological processes in the fetus. Analysis of the fECG already plays a role in intrapartum management through the use of ST analysis (STAN), although this uses the fECG waveform to look at the ST and T waves, rather than assessing fetal HRV (168). Recently, linear and non-linear methods of time series analysis such as spectral analysis and entropy measures have been applied to the analysis of HRV and have proven to be useful in the detection of abnormal cord gases in FGR infants (169).

Phase-rectified signal averaging (PRSA) is a method of analysing the HRV, which has shown success as a prognostic factor to predict survival after myocardial infarction in adults (170). It represents the speed at which the heart rate speeds up and slows down: the average acceleration / deceleration capacity (AAC / ADC) (171). PRSA analysis of the fECG has successfully discriminated between FGR and appropriately grown fetuses prior to 34 weeks' gestation (172), with FGR fetuses showing lower AAC and ADC than their gestation-matched controls (173). This difference could highlight altered cardiovascular function and autonomic control of the heart as a consequence of the chronic hypoxia associated with early-onset FGR.

Monica AN24 records the FHR every 250 milliseconds; this level of accuracy combined with its long-term monitoring capability and the potential to extract ECG data from the trace provides an exciting new method of gaining insight into antenatal FHR patterns and different gestations and in different pregnancy pathologies including eFGR. Used in conjunction with ultrasound findings, this information could be used to better identify fetal compromise and better time delivery in growth-restricted infants.

1.6. Defining the research question

Although the focus of several research studies over the past years, further clinical research is required within the field of eFGR in order to both unify and improve the clinical management of this condition and provide better information for parents of affected babies.

Defining the incidence of this condition will allow a better understanding of the impact that it has on resources. This is important not only from the point of view of an obstetrician, who will need to

know how frequently high-intensity antenatal resources will need to be directed towards this type of case, but also from the point of view of a neonatologist and paediatrician, who will take over the patient's journey following delivery, and in some cases can be involved in the provision of care for the duration of the child's life. From a research perspective, knowing the incidence of this condition and its associated outcomes will show the impact that improving knowledge in this area will have, and will help direct future funding. Investigating survival in eFGR will provide information that can be used both from a counselling perspective and from a clinical perspective to aid in the timing of delivery. Survival of neonates born at extremely preterm gestations has improved over the past 20 years, particularly in infants born before 25 weeks, but the majority of studies consider outcomes defined by gestation, rather than birthweight, and the confounding effect of eFGR is unclear.

The ability to better predict pregnancy outcome in this cohort of patients will alter both antenatal management and the counselling offered to parents. Ultrasound is currently the most widely used tool to determine fetal wellbeing antenatally, through assessment of fetal growth and Doppler parameters, but there is a need for more data to suggest how these parameters may be used to better inform us as to the perinatal prognosis. In addition, identifying markers of prognosis will improve our knowledge of eFGR and could highlight additional areas of interest that may reveal more about potential alternative management strategies for this condition.

Although FHR monitoring and cCTG are widely used, there remain many unanswered questions, particularly in the context of eFGR and longer-term monitoring patterns. Within eFGR, cCTG is an established monitoring tool, but the usefulness of analysing the cCTG over more than the standard 30-60 minutes remains to be seen. It may be that longer term patterns reveal more information about fetal wellbeing compared to a shorter snapshot, and that this can also be related to prognosis.

Forming a validated management strategy for eFGR should lead to improved outcomes in this cohort of patients, and ultimately could lead to a reduction in the stillbirth rate.

Hypothesis

In cases of eFGR, antenatal factors, ultrasound biometry and Doppler measurements and FHR patterns can be used to better predict outcomes.

Aims

1. To produce data on the current incidence of eFGR locally and nationally and survival by gestation and birthweight.
2. To explore how currently available antenatal factors, including ultrasound parameters, relate to pregnancy outcomes and investigate whether statistical modelling can provide more reliable prognostic information in cases of eFGR.
3. To prospectively examine the validity and potential value of ambulatory 24-hour fetal heart rate monitoring in eFGR to determine if this technique can reveal useful clinical information that can be incorporated into a prognostic model.

Objectives

1. Analyse local data from a tertiary maternity unit, and national data from NHS Scotland to determine the incidence of eFGR and use these data to calculate survival rates by gestation and birthweight (Chapter 2).
2. Perform a retrospective cohort study of cases of eFGR to create a detailed database of antenatal and ultrasound data collected through the course of the pregnancy (Chapter 3).
3. Apply statistical modelling techniques such as logistic regression to the information in the database to determine what factors are important in predicting pregnancy outcome and how these relate, to create a statistical model to allow prediction of pregnancy outcome based on antenatal status (Chapter 3).
4. Use of retrospective cohort to determine how eFGR management has changed over time at a large tertiary maternity unit (Chapter 4)
5. Prospectively recruit 30 eFGR and 30 low-risk pregnancies prior to 32 weeks' gestation to perform 24 hour fetal heart rate monitoring (Chapter 5).
6. Use application of basic statistical comparisons and non-linear time series analysis techniques to determine if patterns of fetal heart rate changes relating to disease or prognosis can be identified (Chapter 5).

CHAPTER 2: INCIDENCE AND NEONATAL SURVIVAL IN PREGNANCIES AFFECTED BY eFGR BETWEEN 2012-2017

2.2. Introduction

eFGR is rarely reported separately from FGR as a whole, therefore the precise incidence of eFGR is currently unknown. As previously mentioned, the incidence of preterm pre-eclampsia, which is estimated to be 0.3% (26), can be used as an estimation given that the two conditions often co-exist (25), but this does not provide an accurate figure. Without this information, it is difficult to appropriately direct both research and clinical funding, as the level of the impact on obstetric care and outcomes remains unclear. The initial aim of this project was to determine the incidence of eFGR on a national scale, and then on a local level in a tertiary unit which receives eFGR referrals from a single NHS region. Following on from this, the project subsequently aimed to explore the relationship between gestational age at delivery and birthweight and neonatal survival. There have been two large studies over previous years to investigate survival in pre-term births (64–72,174). EPICure investigated outcomes up to 16 years of age in infants born across England before 26^{+0} weeks' gestation in 1995, then again in 2006 (66,68), whereas Epipage was performed in France in 1997 and 2011, and included births up to 32 weeks' gestation (70,174). Both studies provided vital information on the relationship between gestational age at birth and survival but did not consider survival in the context of birthweight or FGR. Research by The Infant Mortality and Morbidity Studies Group at The University of Leicester used gestational age at delivery and birthweight as predictors of survival, and this highlighted how survival is affected by birthweight at a given gestation, but not specifically in the context of FGR (73). This study aims to use population data from NHS National Services Scotland (NSS), and a matched local dataset from St Mary's Hospital, Manchester for comparison. It is hoped that by using population and local data to investigate this relationship with a focus on eFGR, the effect of eFGR status on survival, if any, will be better understood. Ultimately, furthering knowledge surrounding survival statistics in eFGR will improve the counselling of affected parents, and it will aid in the decision-making surrounding timing of delivery for parents, obstetricians and neonatologists.

2.2 Methods

The data collection process relating to both the national and local datasets is presented in the following section. The analytical approach that was applied to both datasets is subsequently discussed in Section 2.2.2.

2.2.1. Data acquisition

2.2.1.1. NHS National Services Scotland

Permission was obtained through the NHS Scotland Public Benefit and Privacy Panel for Health to allow access to the Information Services Division of NHS National Services Scotland (NSS), Scottish Morbidity Record (SMR) 02 maternity dataset and the National Records of Scotland (NRS) Births, Stillbirths and Infant Deaths database (project reference: 1819-0089). Data were remotely accessed and analysed through the NSS National Safe Haven. Research ethics approval was not

sought for this part of the project as it was deemed to be covered by the NSS generic ethical approval already in place.

SMR02 is a data source of obstetric outcomes collected from hospital records following an episode of obstetric care (177). Data collected consistently since 1975 include:

- **Mother:** age, height, weight, smoking history, previous obstetric history
- **Baby:** birthweight, gestational age, sex, Apgar score
- **Birth:** mode of delivery, induction, analgesia, outcome

The NRS Births, Stillbirths and Infant Deaths database catalogues all stillbirths and infant deaths in Scotland since 1974, and includes information relating to age at death and cause of death.
Data were requested from both datasets, as following an initial extraction and preliminary data exploration, it transpired that the pregnancy outcome data contained within SMR02 can be inconsistently recorded compared to that recorded in the NRS dataset, so cases were initially identified through the SMR02 dataset, then outcome data cross-checked using the community health index (CHI) number and unique patient identifier (UPI) with the NRS dataset to ensure accuracy of recording of the pregnancy outcome. The total number of births in Scotland for the time period specified was obtained through publicly available data from the National Records of Scotland (https://www.nrscotland.gov.uk/statistics-and-data/statistics/statistics-by-theme/vital-events/births/births-time-series-data; last accessed 16th April 2021).

Data were requested for all singleton births from 1st January 2012 to 31st December 2017 prior to 33 weeks' gestation. Cases were excluded by NHS NSS if they were multiple births or those involving a known fetal abnormality. The data variables requested for each case are summarised in Table 2.1. The following antenatal maternal diagnoses which are known to be associated with an increased risk of eFGR were identified through the use of ICD-10 codes: pre-existing hypertension (O10), pre-existing hypertension with pre-eclampsia (O11), gestational hypertension (O13), pre-eclampsia (O14), eclampsia (O15), unspecified maternal hypertension (O16).

Table 2.1: Data variables requested for each eligible case dataset from 2012-2017.

SMR02 dataset			**NRS Births, Stillbirths, Infant Deaths dataset**	
Maternal data	**Birth data**	**Infant data**	**FDIU**	**NND/Infant death**
Height (cm) / weight (kg) at booking Ethnicity Parity Number of previous FDIU/NND Antenatal maternal diagnoses (e.g. pre-eclampsia) Administration of antenatal steroids CHI number, UPI number*	Year of delivery Mode of delivery	Gestational age at delivery (completed weeks) Birthweight (g) Sex Pregnancy outcome (live birth, still living/FDIU/NND) CHI number UPI number*	Infant CHI Mother UPI Cause of death Birthweight	Infant UPI Mother UPI Cause of death Age at death

*CHI/UPI number requested for purposes of data linkage across the two datasets by NHS Scotland, this data was removed from the dataset before it was uploaded to the National Safe Haven for analysis to maintain patient anonymity. NND, neonatal death.

Following data acquisition, cases with a missing or incongruent birthweight (+/- 5 standard deviations from the gestational age mean) or missing gestational age at delivery were excluded, as both of these variables were required for identification of eFGR cases. Maternal height and weight data were checked for outliers and any incongruent values removed from the dataset. Ethnicity was coded as White British, Asian, African, Black Caribbean, Mixed, Other or Unknown. ICD-10 codes were used to identify those cases also complicated by pre-eclampsia/eclampsia/chronic hypertension. Birthweight centiles were calculated according to Hadlock et al. (75) using gestational age at delivery in completed weeks, as this is the level of detail recorded in the SMR02 and NRS datasets. A cut off of <3rd centile (according to Hadlock (75)) was chosen to define eFGR (8). Infants with a birthweight between 3rd-10th centile were not classed as eFGR as this dataset did not provide fetal Doppler measurements.

2.2.1.2. Local data – St Mary's Hospital, Manchester, UK

Ethical approval was obtained from the Health Research Authority and West Midlands Research Ethics Committee (IRAS ID: 248646; REC reference: 19/WM/0023), and all work was conducted in accordance with the Declaration of Helsinki 1975 (revised 2013). The CMiS maternity database (HD Clinical, Bishops Stortford, Hertfordshire, UK) was used to identify cases at St Mary's Hospital, Manchester, UK which could be used as a local cohort for comparison with the NHS NSS dataset. St Mary's Hospital is a large tertiary maternity unit with level 3 neonatal care in the North-West of England, serving an ethnically and socially diverse population.

Data were extracted for all singleton births prior to 33^{+6} weeks' gestation between 1st January 2012-31st December 2017. Cases coded as multiple births or known fetal abnormalities were excluded. Data collected from each case are summarised in Table 2.2.

Table 2.2: Data variables collected for each eligible case from St Mary's Hospital database.

Maternal data	**Birth data**	**Infant data**
Booking height (cm)	Year of delivery	Gestation at delivery (weeks + days)
Booking weight (kg)	Mode of delivery	Birthweight (g)
Ethnicity		Infant sex
Parity		Pregnancy outcome (live birth, still living/FDIU/neonatal or infant death)
Previous FDIU/neonatal or infant death		

Gestational age at delivery was available to the exact day in the local dataset, therefore this data was recorded. Data obtained from NHS NSS however only specify gestation at delivery in terms of completed weeks, therefore a delivery at 33 weeks and 6 days would be coded as 33 weeks'; to ensure comparable data from St Mary's Hospital all deliveries up to 33^{+6} were therefore included.

As with the Scottish dataset, cases were excluded if they had a missing or incongruent birthweight (+/- 5 standard deviations from the gestational age mean) or missing gestational age at delivery. Maternal height and weight data were checked for outliers and any incongruent values removed from the dataset. Ethnicity was recoded as White British, Asian, African, Black Caribbean, Mixed, Other or Unknown for the purposes of comparison with the Scottish dataset. Diagnoses of pre-

eclampsia and chronic hypertension and administration of antenatal steroids were not reliably recorded through the CMiS computer system, so these data were not collected.

2.2.2. Data analysis

The following analysis was performed on both the national and local datasets. Data analysis was performed using Stata/IC Version 14.1 for Windows for the national dataset and Stata/IC Version 15.1 for Mac (178) for the local dataset, and R (176).

The incidence of eFGR was calculated per year and over the 6-year period by calculating the number of cases in the cohort below the 3rd centile as a proportion of the total annual births in Scotland/St Mary's Hospital for that time period.

Maternal demographic and pregnancy outcome data were checked for normality using the Shapiro-Wilk test. Variables were compared between those with a birthweight centile $\geq$3rd centile and those with a birthweight <3rd centile using Mann Whitney, Chi-squared or Fisher's exact test as appropriate. Univariable analysis was used to determine factors predictive of eFGR in the Scottish dataset and investigate the relationship between the presence of eFGR and maternal disease.

2.2.2.1. Predicting survival – model development

Using the Scottish dataset, univariable analysis was performed using a generalised linear model approach assuming a binomial family with logistic link to predict neonatal death, whereby a live birth which survived to discharge was assigned 0 and neonatal death assigned 1. Variables used as predictors were gestational age at delivery and birthweight. A simple linear relationship was investigated as the initial starting point, then higher order polynomials were included. The likelihood ratio test was used, and P values compared to determine the type of regression that best fitted each variable. If the P value for either the higher order polynomial or the likelihood ratio test was statistically significant, this was taken to indicate an improvement in model fit. Data are shown graphically for each predictor variable, with both raw data and predicted values with the 95% confidence interval.

Multivariable analysis to predict outcome using both gestation and birthweight was also performed. As with the univariable analysis, variables were first included in a simple linear model, before higher order polynomial terms were included. Models were evaluated with and without the inclusion of eFGR status as an interaction term. As previously, P values were interrogated, and the likelihood ratio test used to assess model performance with the addition of the extra parameters and polynomial terms. Once the best fitting model was identified, regression diagnostics were performed.

2.2.2.2. Regression diagnostics

Prior to relying on a statistical model to draw any conclusions or to predict outcomes, model specification should be checked by assessing model fit and identifying observations with a significant impact on coefficient estimates.

The first step is to detect whether or not there is a specification error. When building a logistic regression model, it is assumed that the logit of the dependent variable is a linear combination of the independent variables, and that all relevant variables are included in the model. A specification error arises if the logit is not the correct choice of link function or if the relationship between the dependent and independent variables is not linear. A link test uses the linear predicted value and the linear predicted value squared to rebuild the model.

Secondly, goodness-of-fit is assessed by the Hosmer-Lemeshow test. This test splits the sample observations into x groups according to their predicted probabilities to determine if the observed event rates match the expected events rate in the sample subgroups. A P value < 0.05 implies poor model fit. The main limitation with this test is selecting the correct number of subgroups: a small number of subgroups means the test has less opportunity to detect misspecification whereas a large number of subgroups will result in smaller numbers in each subgroup, which may be too small to detect subtle differences.

Thirdly, any observations with a significant effect on the model coefficients need to be identified. These may represent data entry errors or may be outliers requiring removal from the model. Pearson residuals are calculated as the standardised difference between the observed and predicted frequency. Deviance residuals measure the difference between the maximum of the observed and fitted log likelihood functions. Finally, the hat diagonal is measured as the diagonal of the hat matrix and represents the leverage of each value. The hat matrix maps the vector of the dependent variable values to the vector of the predicted variable values to describe the influence each response value has on the fitted value for that same observation. Each residual is either plotted against predicted probability or in an index plot against case numbers to identify outlier values. Following identification of outlier values, these should be examined for potential data entry error, and the logistic regression rerun without these values to determine if model performance is affected.

For the final model, reported results are presented as odds ratios (95% CI) and coefficients (95% CI). Interval validation of the model was performed using bootstrapping, and the area under the curve (AUC) calculated using a receiver operating characteristic (ROC) curve. A cut off value was also applied, above which the predicted outcome was death following live birth, and below which the predicted outcome was survival. This cut off value was selected by graphing sensitivity and specificity against probability cut off. Using this method, sensitivity, specificity, positive and negative likelihood ratios (+LR/-LR) were calculated to evaluate model performance.

2.2.2.3. External validation

The local St Mary's Hospital dataset was used as a test cohort of pregnancies to externally validate the model coefficients. These test data were used to calculate predicted probabilities using the coefficients from the developed national dataset model. A confusion matrix was generated to identify the number of correctly/incorrectly identified cases, and from this, sensitivity, specificity,

+LR/-LR and the AUC for the test cohort generated to determine how the model performs in an external cohort.

2.2.2.4. Predicting survival

To create a figure for survival based on birthweight and gestation, the resulting coefficients from the best fitting logistic regression model were used. Probability responses (and 95% CI) were estimated at fixed gestational age (24-33 weeks; increments of one week) and birthweight (400-2600g; increments of 200g) intervals.

2.3. Results

2.3.1. Final cohort for analysis

2.3.1.1. Scotland

Exclusion of cases which did not meet the inclusion criteria are shown in the STROBE diagram illustrated by Figure 2.1.

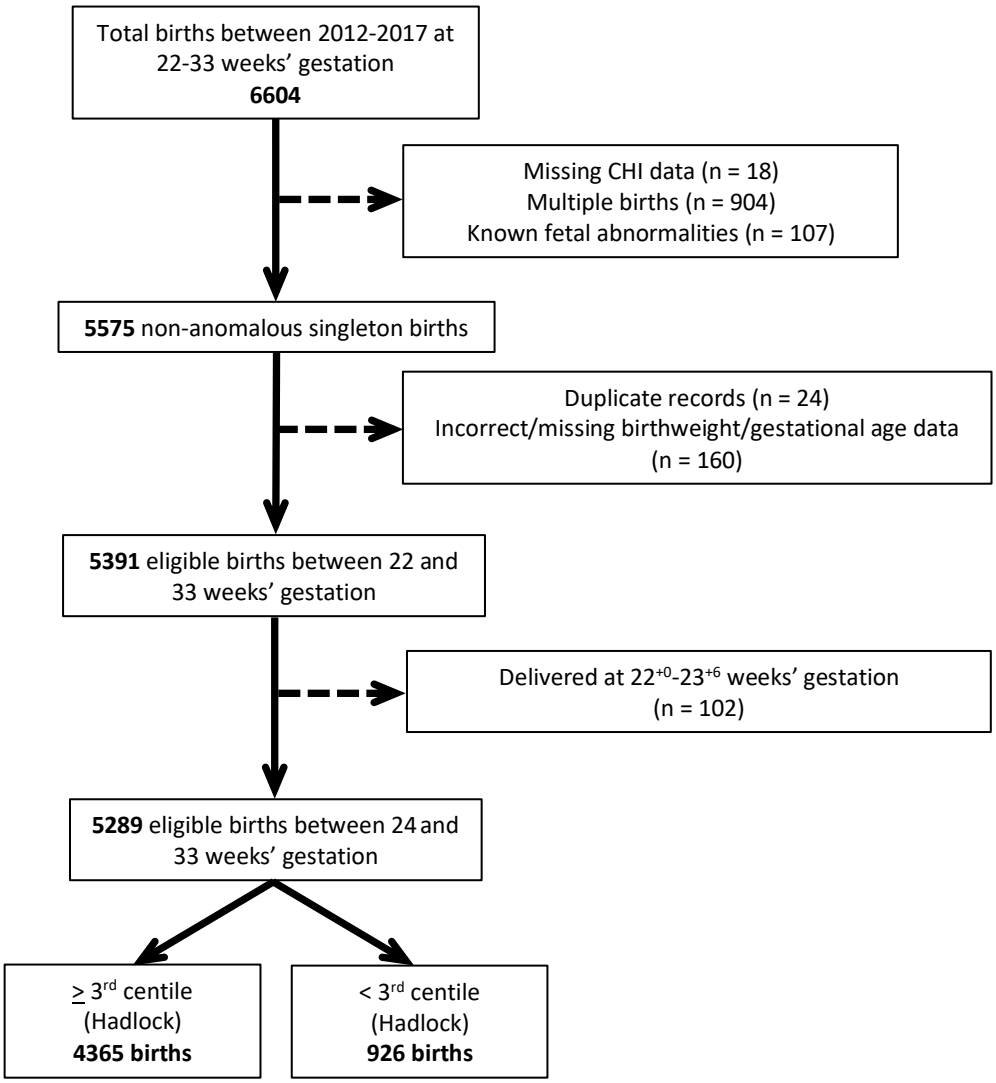


Figure 2.1: STROBE diagram to illustrate exclusion of cases and formation of final cohort for analysis for NHS Scotland database.

For the purposes of the analysis, births that were recorded at 22-23 weeks' gestation were excluded, due to potential inconsistencies in recording of these extremely premature deliveries across different hospitals.

2.3.1.2. St Mary's Hospital, Manchester

Figure 2.2 summarises the number of cases included in the final cohort for analysis for the St Mary's database, and the reason for case exclusion.

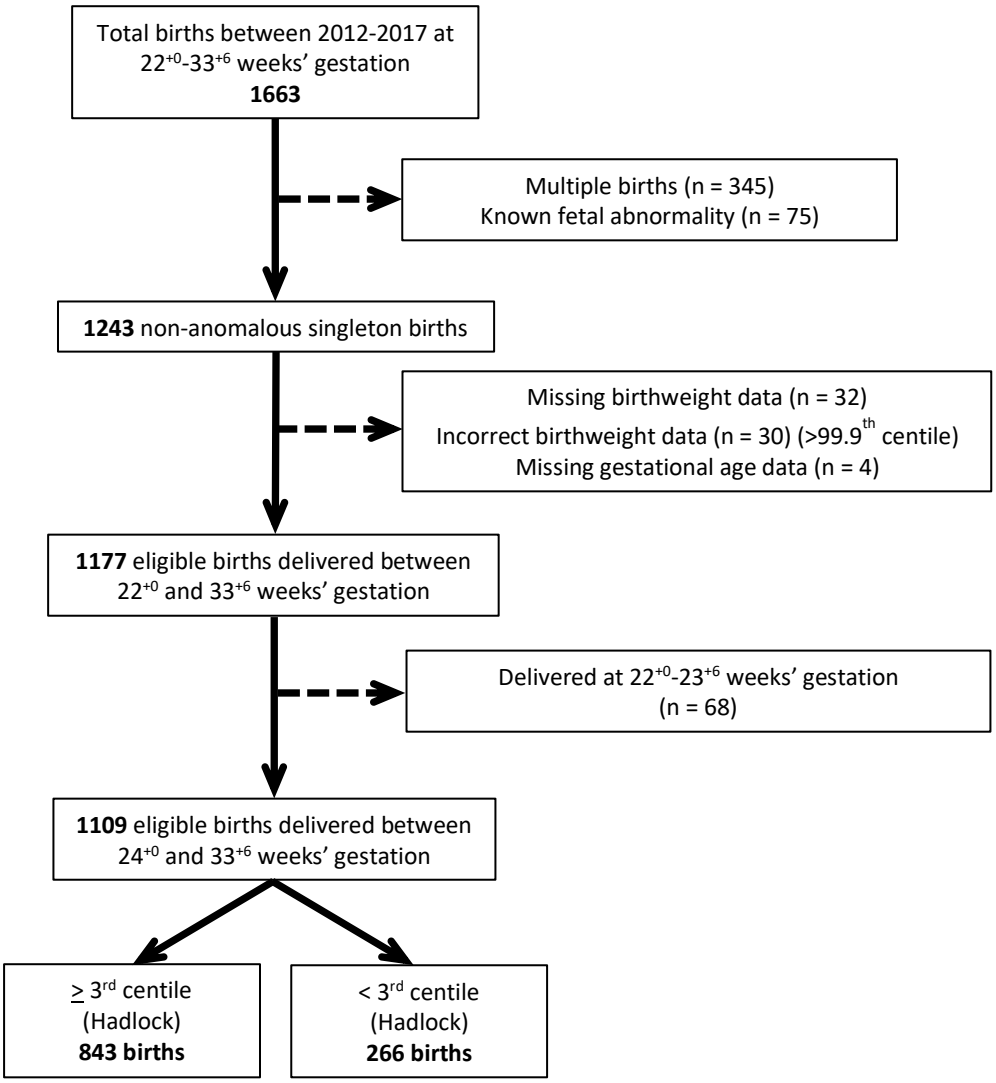


Figure 2.2: STROBE diagram to illustrate exclusion of cases and formation of final cohort for analysis for St Mary's Hospital database.

2.3.2. Incidence of eFGR

2.3.2.1. Scotland

Across the six-year study period (2012-2017), there were 5289 eligible singleton, non-anomalous deliveries between 24 and 33 completed weeks' gestation. 926 cases had a birthweight <3rd centile for gestational age and hence were classified as eFGR. Incidence of eFGR was estimated by

identifying the eligible cases with a birthweight < 3rd centile for gestation. The number of cases per year, and the annual incidence with 95% confidence intervals are summarised in Table 2.3.

Table 2.3: eFGR incidence for each year of the study period (2012-2017) and across the whole period for the whole of Scotland.

Year	Total number of births	Number of eFGR cases	Estimated incidence of eFGR per 1000 births (95% confidence interval)
2012	58,027	154	2.7 (2.3-3.1)
2013	56,014	144	2.6 (2.2-3.0)
2014	56,725	155	2.7 (2.3-3.2)
2015	55,098	154	2.8 (2.4-3.3)
2016	54,488	169	3.1 (2.7-3.6)
2017	52,861	150	2.8 (2.4-3.3)
Total	**333,213**	**926**	**2.8 (2.6-3.0)**

Figure 2.3 shows how this rate has behaved over the study period. From our data, it can be concluded that the incidence of eFGR is approximately 3 per 1000 births in the Scottish population.

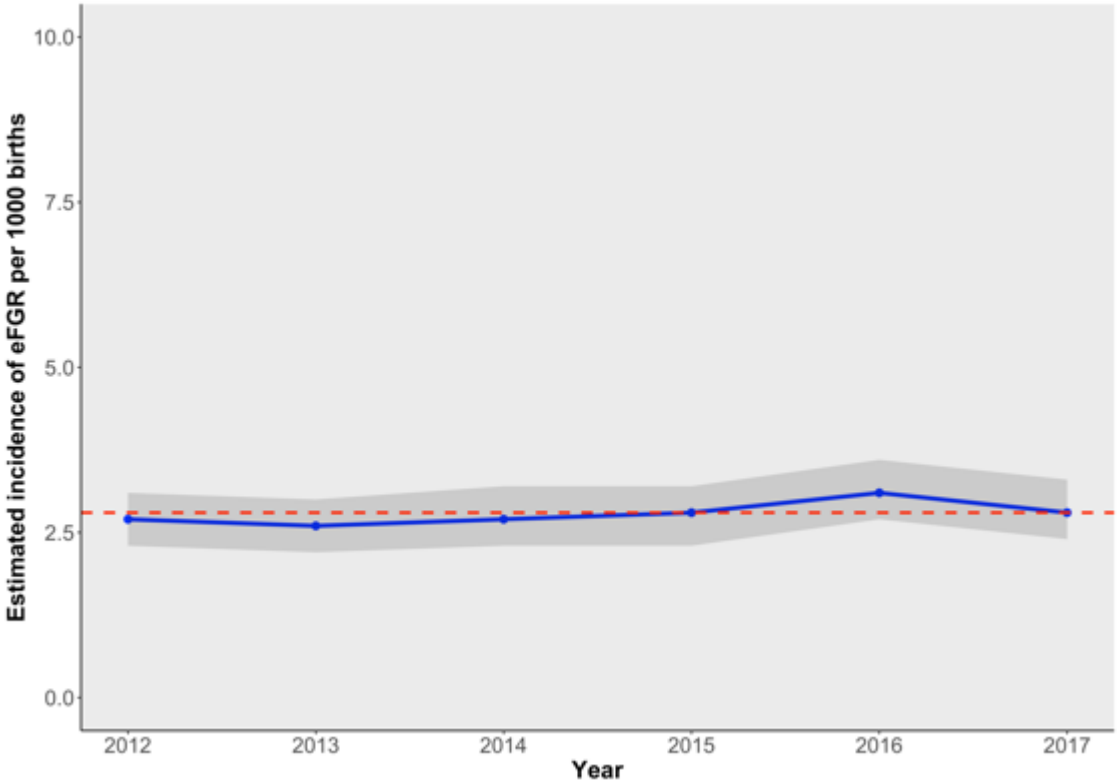


Figure 2.3: Line graph to show yearly incidence of eFGR across the study period (2012-2017) with calculated rate (blue line) and 95% confidence interval (grey area). The red dashed line denotes the incidence for the overall study period (2.8 cases per 1000 pregnancies).

2.3.2.2. St Mary's Hospital, Manchester

From 2012-2017, there were 54,726 births at St Mary's Hospital, of which 1177 were singleton, non-anomalous deliveries between 24 and 33 weeks' gestation, with complete birthweight and gestational age data. An additional 68 births were identified as delivered at 22^{+0}-23^{+6} weeks gestation, but as deliveries at these early gestations were not included in the larger Scottish cohort,

they were excluded from the analysis of the St Mary's cohort which required direct comparison with the Scottish cohort. The number of cases per year, and the annual incidence with 95% confidence intervals are summarised in Table 2.

Table 2.4: eFGR incidence for each year of the study period (2012-2017) and across the whole period for the St Mary's Hospital population.

Year	Total number of births	Number of eFGR cases	Estimated incidence of eFGR per 1000 births (95% confidence interval)
2012	8214	41	5.0 (3.6-6.8)
2013	8925	43	4.8 (3.5-6.5)
2014	9152	44	4.9 (3.5-6.4)
2015	9406	60	6.4 (4.9-8.2)
2016	9603	33	3.5 (2.4-4.8)
2017	9426	45	4.8 (3.5-6.4)
Total	**54,726**	**266**	**4.9 (4.3-5.5)**

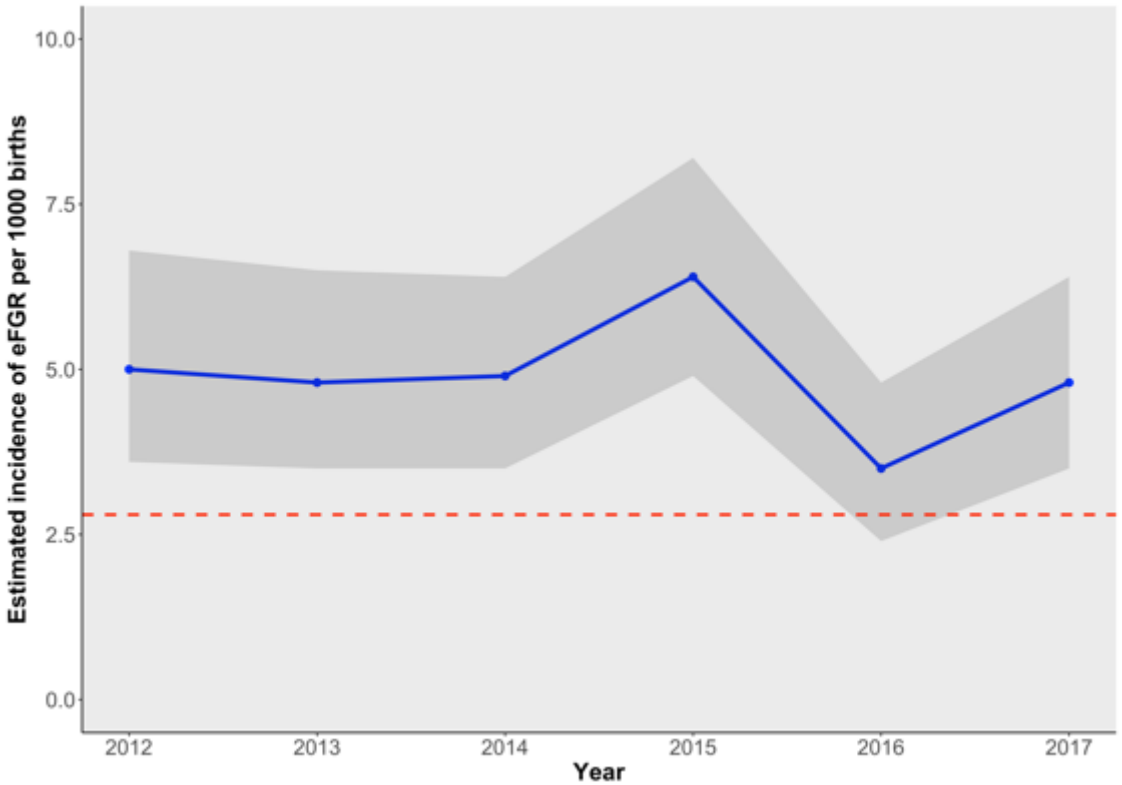


Figure 2.4: Line graph to show yearly incidence of eFGR specific to St Mary's Hospital across the study period (2012-2017) with calculated rate (blue line) and 95% confidence interval (grey area). The red dashed line denotes the national incidence for the overall study period as calculated from the Scottish data set, as detailed in Section 2.3.2.1. (2.8 cases per 1000 pregnancies).

Figure 2.4 depicts the change in rate across the study period; the rate of eFGR pregnancies in the St Mary's population is approximately 5 per 1000 pregnancies, although there was an increase in the rate in 2015, subsequently followed by an apparent decrease.

2.3.3. Demographic data

2.3.3.1. Scotland

The maternal characteristics of the study population are summarised in Table 2.5. There was a statistically significant difference between eFGR and non-eFGR cases in terms of maternal height ($P < 0.001$), although this is not likely to be of any clinical significance. Parity was also significantly associated with eFGR, with 56.5% of eFGR cases in primiparous women, compared to 46.8% of non-eFGR cases ($P < 0.001$).

Table 2.5: Summary of maternal characteristics for non-eFGR and eFGR cases included in the final analysis of the NHS Scotland population data.

Variable	Non-eFGR cases (n = 4365)	eFGR cases (n = 926)	Significance
Maternal height (cm)*	163 ± 6.56	163 ± 6.60	P < 0.001
Maternal weight (kg)*	70.8 ± 17.4	71.9 ± 18.1	NS P = 0.10
Ethnicity†			
White	3106 (71.2%)	633 (68.4%)	
Asian	131 (3.0%)	40 (4.3%)	
African	56 (1.3%)	19 (2.1%)	NS P = 0.11
Black/Caribbean	13 (0.3%)	1 (0.1%)	
Arabic	22 (0.5%)	4 (0.4%)	
Mixed	11 (0.3%)	4 (0.4%)	
Other/unknown	1026 (23.51%)	225 (24.3%)	
Parity†			
Primiparous	2041 (46.8%)	523 (56.5%)	P < 0.001
Multiparous	2324 (53.2%)	403 (43.5%)	

*Mean±SD, t-test; †Counts (percentage), Chi-squared test

Pregnancy outcome data for the cohort are summarised in Table 2.6. All parameters were significantly different across the two groups, with the eFGR cases unsurprisingly more likely to be born at an earlier gestation ($P < 0.001$), and with a lower birthweight ($P < 0.001$). The distribution of infant sex differed between the two groups, with the eFGR cases having a higher proportion of females (53.4%) and the non-eFGR cases having a higher proportion of males (58.0%) ($P < 0.001$). eFGR cases had a different spread of outcomes, with more cases ending in FDIU (21.4% compared to 6.2%) and neonatal/infant death (6.2% compared to 4.7%) compared to the non-eFGR cases ($P < 0.001$). Fewer patients in the eFGR group received antenatal steroids ($P < 0.001$) (64.3% compared to 71.5% in non-eFGR). There was a higher rate of delivery by Caesarean section in the eFGR cases ($P < 0.001$) (72.2% compared to 52.3% in non-eFGR cases).

Table 2.6: Summary of pregnancy outcome data for non-eFGR and eFGR cases included in the final analysis of the NHS Scotland population data.

Variable	Non-eFGR cases (n = 4365)	eFGR cases (n = 926)	Significance
Gestational age at delivery (completed weeks)*	31 (24-33)	31 (24-33)	P < 0.001
Birthweight (g)*	1680 (505-3000)	1040 (320-1620)	P < 0.001
Mode of delivery†			
Vaginal	2076 (47.6%)	254 (27.4%)	P < 0.001
Caesarean section	2283 (52.3%)	669 (72.2%)	
Unknown	6 (0.1%)	3 (0.3%)	
Outcome†			
Live birth, still alive	3890 (89.1%)	670 (72.4%)	P < 0.001
FDIU	269 (6.2%)	198 (21.4%)	
Neonatal death	206 (4.7%)	58 (6.2%)	
Antenatal steroids†			
Yes (1 or 2 doses)	3119 (71.5%)	595 (64.3%)	P < 0.001
No	486 (11.1%)	163 (17.6%)	
Not recorded	760 (17.4%)	168 (18.1%)	
Infant sex†			
Male	2533 (58.0%)	425 (45.9%)	P < 0.001
Female	1829 (41.9%)	494 (53.4%)	
Unknown/not recorded	3 (0.1%)	7 (0.8%)	

*Median (range), Mann-Whitney test; †Counts (percentage), Chi-squared test

The incidence of hypertensive disease also differed between the two groups; eFGR cases were approximately three times more likely to have a diagnosis of pre-eclampsia during pregnancy (15.6% compared to 4.9%; P < 0.001), or to have pre-existing renal or hypertensive disease (3.1% compared to 1.0%; P < 0.001). Preterm prelabour rupture of membranes (PPROM) occurred more frequently in the non-eFGR cases compared to the eFGR cases (19.7% compared to 5.0%; P < 0.001). These data are summarised in Table 2.7.

Table 2.7: Maternal disease in the study population included in the final analysis of the NHS Scotland population data.

Maternal condition	Non-eFGR cases (n = 4365)	eFGR cases (n = 926)	Significance
*Pre-eclampsia**			
No	4152 (95.1%)	782 (84.5%)	P < 0.001
Yes	213 (4.9%)	144 (15.6%)	
*Pre-existing CKD/HTN**			
No	4322 (99.0%)	897 (96.9%)	P < 0.001
Yes	43 (1.0%)	29 (3.13%)	
*PPROM**			
No	3505 (80.3%)	880 (95.0%)	P < 0.001
Yes	860 (19.7%)	46 (5.0%)	

CKD: Chronic kidney disease; HTN: Hypertension; PPROM: Pre-term pre-labour rupture of membranes
*Counts (percentage), Chi-squared test

2.3.3.2. St Mary's Hospital, Manchester

Maternal characteristics of the study population are summarised in Table 2.8. There was no significant difference in terms of maternal height or weight. Ethnicity was significantly different between eFGR and non-eFGR cases, with there being a higher proportion of Asian women in the eFGR compared to the non-eFGR group, and a lower proportion of white women. Parity was also significantly different, with the eFGR group having a higher proportion of primiparous women (56.8% compared to 47.0% in non-eFGR cases).

Table 2.8: Summary of maternal characteristics for non-eFGR and eFGR cases included in the final analysis of the St Mary's Hospital local cohort.

Variable	Non-eFGR cases (n = 843)	eFGR cases (n = 266)	Significance
Maternal height (cm)*	163 (122-184)	162 (144-179)	NS P = 0.06
Maternal weight (kg)*	67 (31-145)	68 (40-130)	NS P = 0.49
Ethnicity†			
White	509 (60.4%)	135 (50.0%)	
Asian	138 (16.4%)	53 (19.6%)	
African	97 (11.5%)	36 (13.3%)	P = 0.02
Black/Caribbean	39 (4.6%)	14 (5.2%)	
Mixed	25 (3.0%)	9 (3.3%)	
Other/unknown	35 (4.2%)	19 (8.5%)	
Parity†			
Primiparous	396 (47.0%)	151 (56.8%)	P = 0.01
Multiparous	447 (53.0%)	115 (43.2%)	

*Median (range), Mann-Whitney test; †Counts (percentage), Chi-squared test

Pregnancy outcome data for the cohort are summarised in Table 2.9. All parameters were significantly different across the two groups, with the eFGR cases more likely to be born at an earlier gestation (P < 0.001), and with a lower birthweight, as by study design. There was a higher rate of delivery by Caesarean section in the eFGR group (64.7% compared to 44.0% in the non-eFGR group; P < 0.001). The distribution of infant sex differed between the two groups, with the eFGR cases having a higher proportion of females (51.1%) and the non-eFGR cases having a higher proportion of males (56.4%) (P < 0.001). eFGR cases had a different range of outcomes, with more cases ending in FDIU (27.4% compared to 6.3%) and neonatal/infant death (9.8% compared to 4.9%) compared to the non-eFGR cases (P = 0.002).

Table 2.9: Summary of pregnancy outcome data for non-eFGR and eFGR cases included in the final analysis of the St Mary's local cohort.

Variable	Non-eFGR cases (n = 843)	eFGR cases (n = 266)	Significance
Gestational age at delivery (completed weeks)*	31 (24-33)	30 (24-33)	P = 0.002
Birthweight (g)*	1556 (520-2746)	852 (210-1618)	
Mode of delivery†			
Vaginal	472 (56.0%)	91 (34.2%)	
Caesarean section	371 (44.0%)	172 (64.7%)	P < 0.001
Unknown	0 (0.0%)	3 (1.1%)	
Outcome†			
Live birth, still alive	749 (88.9%)	167 (62.8%)	
FDIU	53 (6.3%)	73 (27.4%)	P < 0.001
Neonatal death	41 (4.9%)	26 (9.8%)	
Infant sex†			
Male	460 (56.4%)	127 (47.7%)	P = 0.002
Female	383 (45.4%)	136 (51.1%)	
Unknown/not recorded	0 (0.0%)	3 (1.1%)	

*Median (range), Mann-Whitney test; †Counts (percentage), Chi-squared test

2.3.4. Prediction of neonatal death using NHS Scotland population data

The cases used for this section of the analysis involved only those that ended in a live birth, thus the FDIU cases were excluded.

2.3.4.1. Gestational age at delivery

The relationship between survival and gestation at delivery was investigated using logistic regression, with a binomial/link family. It was found that this was best modelled including gestational age as a quadratic term, so the chance of neonatal death reduced as gestation increased (P = 0.03). The risk of death was higher in eFGR cases at any gestation, and the interaction between gestational age and eFGR status was significant (P = 0.004), suggesting that eFGR status influences how this relationship changes with advancing gestation. Regression coefficients for all models investigated are summarised in Table 2.10; model 5 (shaded grey) corresponds to the best fit model (gestational age as a quadratic term and eFGR status as an interaction term).

Table 2.10: Logistic regression model to predict neonatal death based on gestational age at delivery using NHS Scotland population data.

Covariate	Level	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 1				
Gestational age (weeks)		– 0.53 (– 0.58-— 0.47)	0.59 (0.56-0.62)	< 0.001
Constant		12.3 (10.9-13.8)		< 0.001
Model 2				
Gestational age (weeks)		– 2.78 (– 3.85- – 1.71)	0.06 (0.02-0.18)	< 0.001
Gestational age^2 (weeks)		0.04 (0.02-0.06)	1.04 (1.02-1.06)	< 0.001
Constant		43.7 (28.7-58.7)		< 0.001
Model 3				
Gestational age (weeks)		– 15.2 (– 34.3-3.88)	$2.44e^{-7}$($1.22e^{-15}$-48.5)	0.12
Gestational age^2 (weeks)		0.48 (–0.20-1.16)	1.62 (0.82-3.20)	0.16
Gestational age^3 (weeks)		– 0.01 (– 0.01-0.002)	0.99 (0.99-1.00)	0.20
Constant		159 (–18.7-338)		0.08
Model 4				
Gestational age (weeks)		– 3.00 (–4.08- – 1.92)	0.05 (0.02-0.15)	< 0.001
Gestational age^2 (weeks)		0.04 (0.02-0.06)	1.04 (1.02-1.06)	< 0.001
Centile_3	No	Reference	Reference	
	Yes	0.84 (0.49-1.18)	2.31 (1.64-32.6)	< 0.001
Constant		46.8 (31.6-61.9)		< 0.001
Model 5				
Gestational age (weeks)		– 2.34 (– 3.54- – 1.14)	0.10 (0.03-0.32)	< 0.001
Gestational age^2 (weeks)		0.03 (0.01-0.05)	1.03 (1.01-1.06)	0.003
Centile_3	No	Reference	Reference	
	Yes	70.9 (25.1-116.7)	$6.03e^{30}$($7.59e^{10}$-$4.81e^{50}$)	0.002
Centile_3*gestational age		– 4.82 (– 8.01- – 1.62)	0.008 (0.003-0.20)	0.003
Centile_3*gestational age^2		0.08 (0.03-0.14)	1.09 (1.03-1.15)	0.004
Constant		37.4 (20.6-54.2)		< 0.001

Likelihood ratio test:
Model 1 vs Model 2: P < 0.001
Model 2 vs Model 3: P = 0.20
Model 2 vs Model 4: P = 0.001
Model 4 vs Model 5: P =0.03

Figure 2.5 shows how the relationship between gestational age and predicted probability of death changes, and how eFGR status influences this. The predicted probability of neonatal death declines for both eFGR and non-eFGR cases as gestation advances, but there is a statistically significant difference in the likelihood of death between eFGR and non-eFGR cases. eFGR status has a larger impact on survival chances at earlier gestations, but survival chances are similar beyond 28 weeks' gestation.

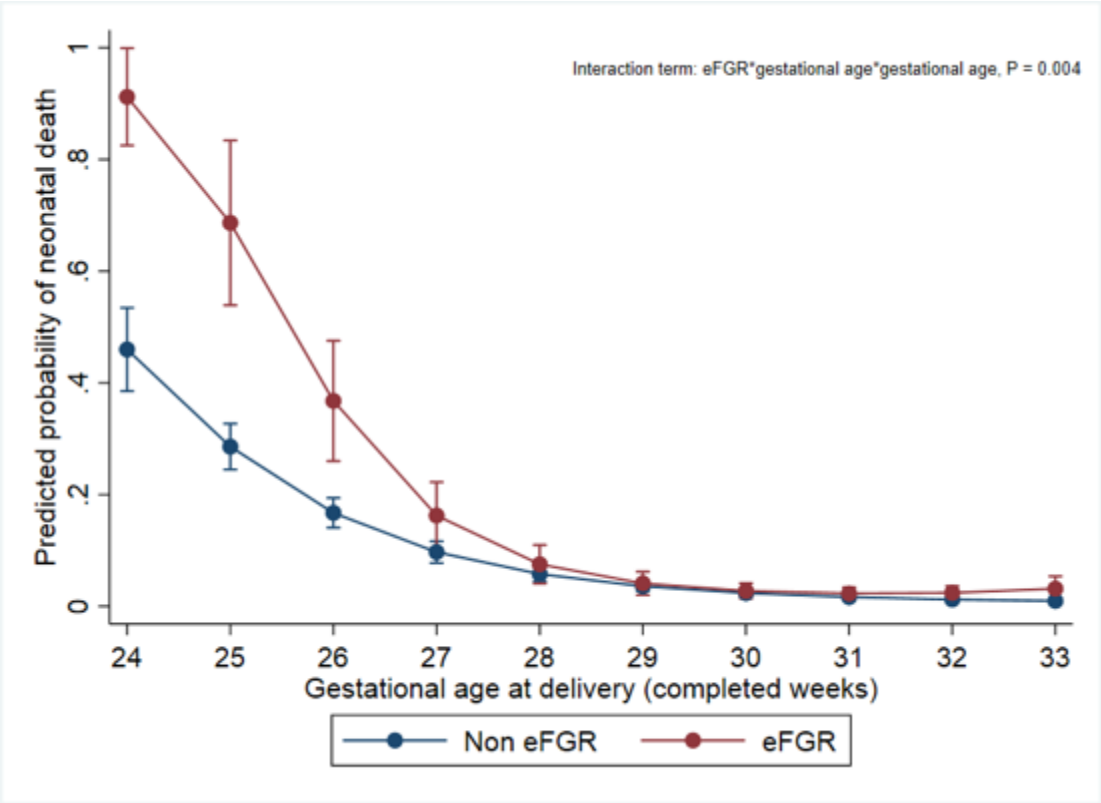


Figure 2.5: Predicted probability of neonatal death by gestational age at delivery for non-eFGR cases (blue) and eFGR cases (red). Based on NHS Scotland population data (n = 5289 births between 24-33 weeks; n = 4365 non-eFGR; n = 926 eFGR).

2.3.4.2. Birthweight

The relationship between birthweight and neonatal death was best modelled using birthweight as a quadratic term (Table 2.11). The risk of neonatal death falls as birthweight increases (P < 0.001) for all cases.

Table 2.11: Logistic regression model to predict neonatal death based on birthweight using NHS Scotland population data. Birthweight is presented in 25g increments.

Covariate	Level	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 1				
Birthweight (g)		– 0.07 (– 0.08-–0.06)	0.93 (0.92-0.94)	< 0.001
Constant		0.82 (0.44-1.21)		< 0.001
Model 2				
Birthweight (g)		– 0.19 (– 0.22- – 0.16)	0.83 (0.80-0.85)	< 0.001
Birthweight^2		0.001 (0.001-0.001)	1.00 (1.00-1.00)	< 0.001
Constant		3.54 (2.79-4.29)		< 0.001
Model 3				
Birthweight (g)		– 0.22 (– 0.33-– 0.13)	0.80 (0.72-0.88)	< 0.001
Birthweight^2		0.002 (– 0.0001-0.004)	1.00 (1.00-1.00)	0.05
Birthweight^3		$3.38e^{-6}$ ($–1.3e^{-5}$-$6.52e^{-6}$)	1.00 (1.00-1.00)	0.50
Constant		4.05 (2.38-5.72)		< 0.001
Model 4				
Birthweight (g)		– 0.21 (– 0.25- – 0.18)	0.81 (0.78-0.83)	< 0.001
Birthweight^2		0.001 (0.001-0.002)	1.00 (1.00-1.00)	< 0.001
Centile_3	No	Reference	Reference	
	Yes	– 1.00 (-1.37- – 0.63)	0.37 (0.25-0.53)	< 0.001
Constant		4.47 (3.64-5.29)		< 0.001
Model 5				
Birthweight (g)		– 0.21 (– 0.25- – 0.18)	0.81 (0.78-0.84)	< 0.001
Birthweight^2		0.001 (0.001-0.002)	1.00 (1.00-1.00)	< 0.001
Centile_3	No	Reference	Reference	
	Yes	1.31 (-1.34-3.97)	3.72 (0.26-53.0)	0.33
Centile_3*birthweight		– 0.14 (– 0.29-0.006)	0.87 (0.75-1.01)	0.059
Centile_3*birthweight^2		0.002 (0.00-0.004)	1.00 (1.00-1.00)	0.04
Constant		4.37 (3.40-5.33)		< 0.001

Likelihood ratio test:
Model 1 vs Model 2: P < 0.001
Model 2 vs Model 4: P < 0.001
Model 4 vs Model 5: P = 0.14

Figure 2.6 shows the change in the predicted probability of neonatal death as birthweight increases. eFGR has a significant impact on predicted outcome, with eFGR infants having a lower predicted probability of neonatal death for any given birthweight.

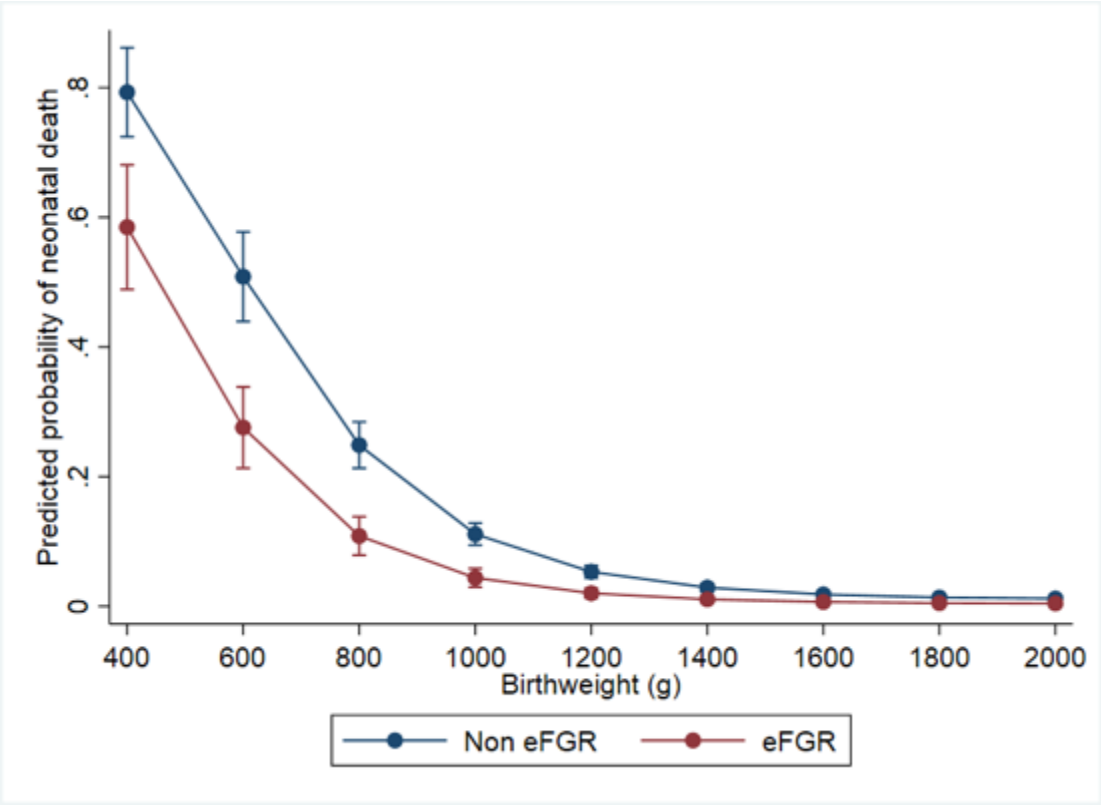


Figure 2.6: Predicted probability of neonatal death by birthweight for non-eFGR cases (blue) and eFGR cases (red). Based on NHS Scotland population data (n = 5289 births between 24-33 weeks; n = 4365 non-eFGR; n = 926 eFGR).

2.3.4.3. Gestational age and birthweight at delivery

Gestational age at delivery and birthweight can also be considered together to predict survival chances. This is best modelled using both gestational age (P < 0.001) and birthweight (P = 0.01) as quadratic terms (Table 2.12). eFGR status when considered as an independent covariate has a significant effect (P = 0.01).

Table 2.12: Logistic regression model to predict neonatal death based on gestational age and birthweight using NHS Scotland population data.

Covariate	Level	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 11				
Gestational age (weeks)		– 2.10 (– 3.37- – 0.82)	0.12 (0.03-0.44)	< 0.001
Gestational age^2 (weeks)		0.03 (0.01-0.05)	1.03 (1.00-1.05)	0.01
Birthweight		– 0.002 (– 0.004-0.000)	0.99 (0.99-0.99)	0.04
Birthweight^2		$7.59e^{-7}$ ($1.93e^{-7}$-$1.33e^{-6}$)	1.00 (1.00-1.00)	0.01
eFGR status	No	Reference	Reference	0.01
	Yes	0.71 (0.16-1.27)	2.04 (1.17-3.56)	
Constant		35.2 (17.7-52.8)		< 0.001

Combining gestational age at delivery and birthweight with eFGR status as a covariate provides a more accurate prediction of survival chances than using either variable alone, as indicated by a significant LR test (P < 0.001). Inclusion of eFGR status as an interaction term did not improve model performance (LR test; P = 0.14). This model has an AUC of 0.88 (95% CI 0.86-0.90). Using

a threshold of ≥ 0.05 to define a neonatal death, test performance statistics are summarised in Table 2.13.

Table 2.13: Summary of test performance for combined gestational age / birthweight NHS Scotland population model.

		Observed outcome		
		Neonatal death	*Survived to discharge*	
Predicted outcome	*Neonatal death*	201	1011	PPV: 16.6%
	Survived to discharge	63	3548	NPV: 98.3%
		Sensitivity: 76.1%	Specificity: 77.8%	
		+LR: 3.56	–LR: 0.27	

PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; – LR: negative likelihood ratio

Internal validation using bootstrapping with 500 replications did not significantly alter model performance (Table 2.14), suggesting that the model is stable, although this would be expected given the size of the dataset.

Table 2.14: Bootstrapping coefficients for combined gestational age / birthweight model

Covariate	Level	Observed bootstrap coefficient (95% confidence interval)	P value
Gestational age (weeks)		– 2.10 (– 3.41- – -0.78)	0.002
Gestational age^2 (weeks)		0.03 (0.01-0.05)	0.019
Birthweight		– 0.002 (– 0.004-0.000)	0.05
Birthweight^2		7.59e^{-7} (2.03e^{-7}-1.32e^{-6})	0007
eFGR status	No	Reference	0.009
	Yes	0.71 (0.17-1.25)	
Constant		35.2 (17.1-53.4)	< 0.001

2.3.4.3.1. Regression diagnostics

Model specification was assessed using the linktest function in Stata 15.0 (178). The linear predicted value reached significance (P < 0.001), but the linear predicted value square did not (P = 0.15), confirming that the logit function was appropriate, and all relevant variables were included in the model. The Hosmer and Lemeshow goodness of fit test suggests the model does not fit the data well (P = 0.03; 10 groups) and a calibration plot (**Error! Reference source not found.**7) highlights that it tends to overestimate predicted values (therefore is more likely to give a false positive result).

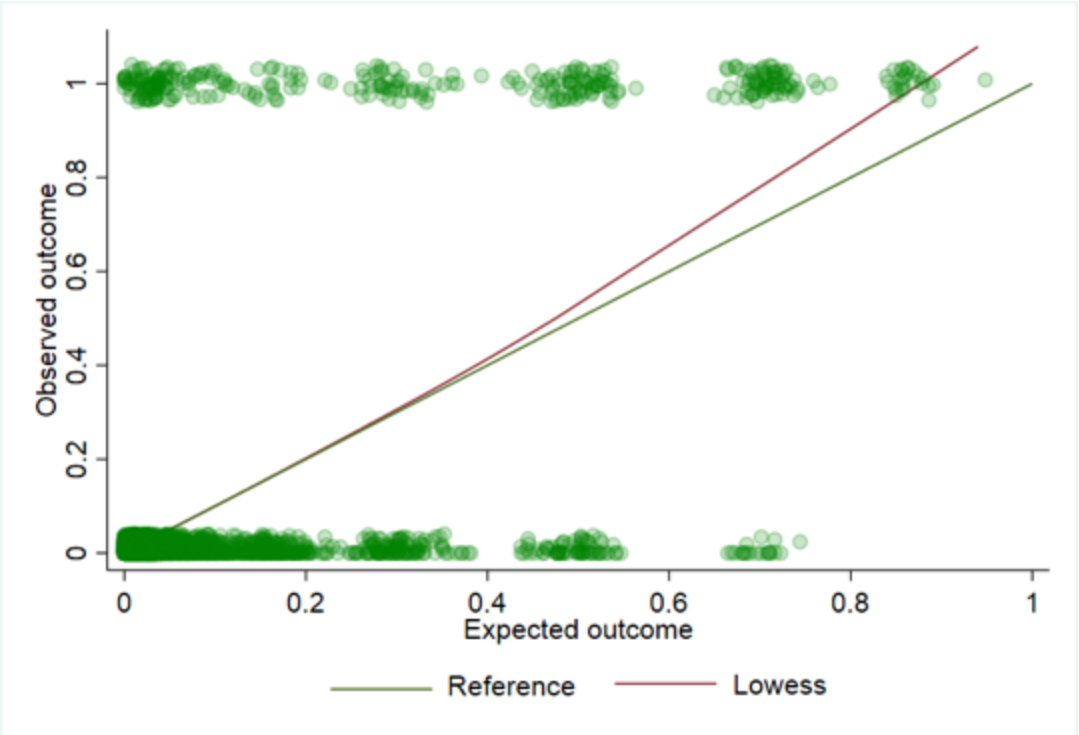


Figure 2.7: Calibration plot to show performance of combined gestational age / birthweight model

Testing for influential observations highlighted that the model coefficients are heavily influenced by a number of observations (Figure 2.8). Influential observations (n = 466) were classified as those with a Pearson residual or deviance value of greater than two, or a leverage value of 3 times the mean leverage. These observations were considered in isolation to determine if they appear to be random outliers. The birthweight, gestational age and pregnancy outcomes for those observations are summarised by Table 2.15.

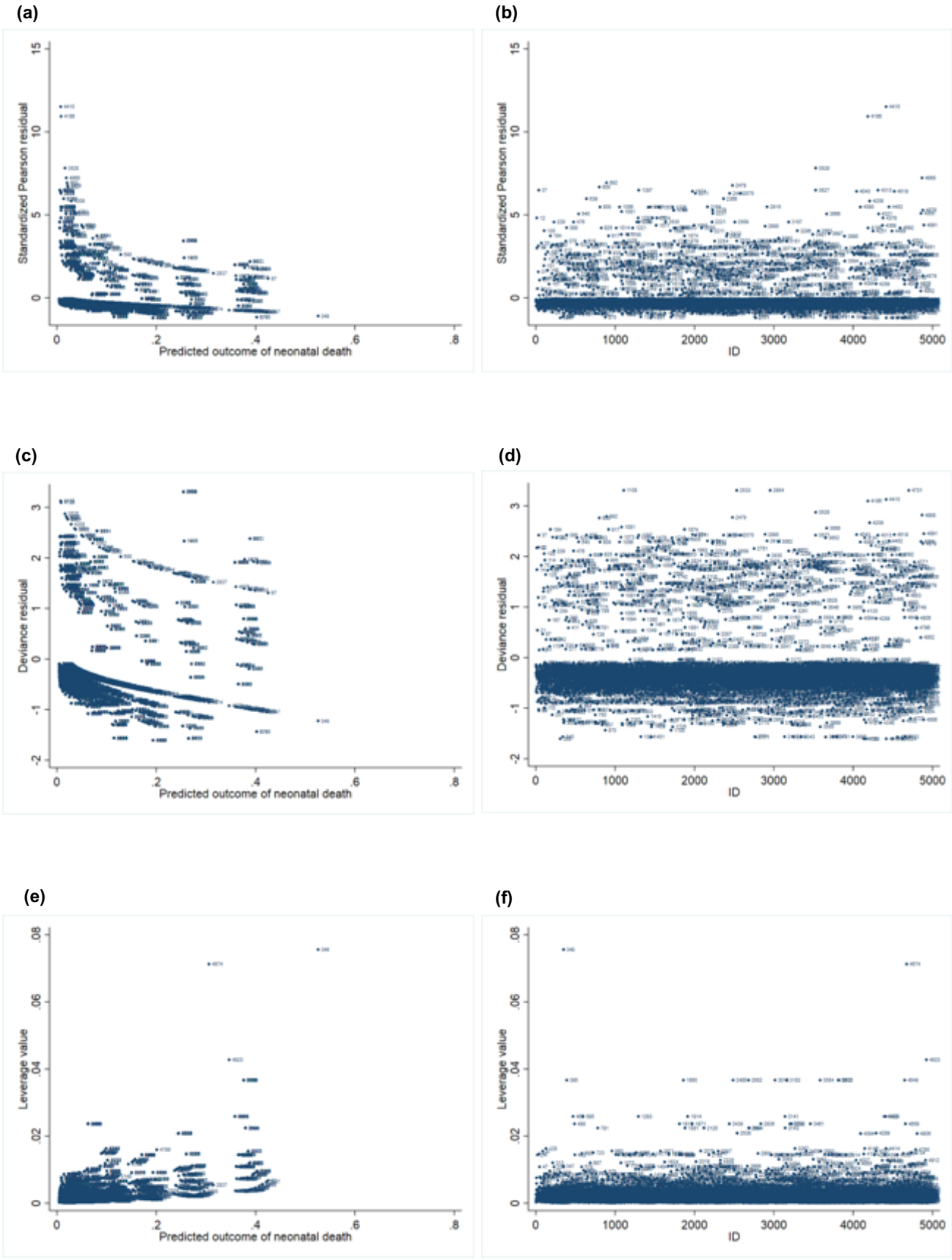


Figure 2.8: Pearson's residuals **(a-b**), deviance residuals **(c-d)** and the leverage of each observation **(e-f)** plotted against predicted probabilities (a, c, e), and as an index plot (b, d, f). Influential observations were deemed to be those with a Pearson residual or deviance value of greater than two, or a leverage value of 3 times the mean leverage.

Table 2.15: Summary of characteristics for influential observations. P value denotes comparison with whole Scottish population and highlights that the influential observations have a higher proportion of eFGR infants and cases that ended in neonatal death.

	Influential values (n = 466)	P value
Gestational age (completed weeks)*	31 (24-33)	0.16
Birthweight (g)*	1627 (300-2790)	0.229
Pregnancy outcome†		
Survived to discharge	253 (68.9%)	< 0.001
Neonatal / infant death	110 (31.1%)	
eFGR†		
No	324 (89.3%)	0.001
Yes	39 (10.7%)	

*Median (range), Mann-Whitney test; †Counts (percentage), Chi-squared test

Although the gestational age at birth and the birthweight are not significantly different in the influential observations, the pregnancy outcomes and eFGR status are. This suggests using the whole population to develop the model leaves it disproportionately influenced by eFGR cases and cases that ended in neonatal death. This suggests that the relationship between gestational age, birthweight and pregnancy outcome cannot be reliably quantified using this dataset. The following section details the relationship between gestational age, birthweight and pregnancy outcome for those births involving a birthweight above 600g.

2.3.4.4. Prediction of neonatal death using gestational age and birthweight > 600g

The logistic regression model to predict the probability of neonatal death based on gestational age and birthweight for births above 600g (n = 4749; non-eFGR cases n = 4078, eFGR cases n = 671) is summarised in Table 2.16.

Table 2.16: Logistic regression model to predict neonatal death based on gestational age and birthweight using NHS Scotland population data for all cases with a birthweight above 600g.

Covariate	Level	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Gestational age (weeks)		– 0.612 (– 0.736- – 0.490)	0.54 (0.48-0.61)	< 0.001
Birthweight (g)		0.0007 (0.0004-0.001)	1.00 (1.00-1.00)	0.037
eFGR status	No	Reference	Reference	0.031
	Yes	0.637 (0.058-1.22)	1.89 (1.06-3.37)	
Constant		13.67 (11.0-16.3)		< 0.001

The Hosmer and Lemeshow goodness of fit test suggests a better fit for this model (P = 0.09; 10 groups), however the calibration plot suggests again that there is overestimation of the probability of neonatal death (Figure 2.9). Testing for influential observations (not shown) again suggests that the model is heavily influenced by those cases ending in neonatal death, and cases of eFGR.

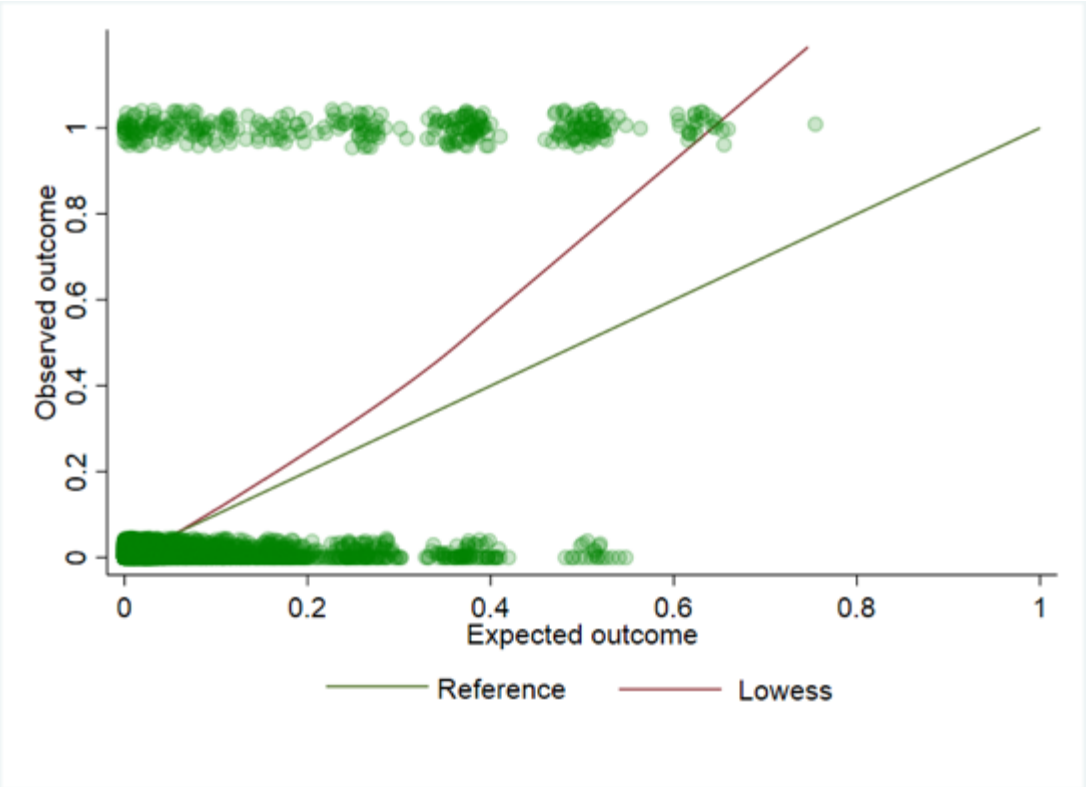


Figure 2.9: Calibration plot to show performance of combined gestational age / birthweight model for births above 600g only.

This model has an AUC of 0.87 (95% CI 0.85-0.90). Using a threshold of ≥ 0.04 to define a neonatal death, test performance statistics are summarised in Table 2.17.

Table 2.17: Test performance for combined gestational age / birthweight model for all births > 600g in NHS Scotland population dataset (n = 4749).

		Observed outcome		
		Neonatal death	*Survived to discharge*	
Predicted outcome	*Neonatal death*	167	1113	PPV: 13.05%
	Survived to discharge	55	3414	NPV: 98.4%
		Sensitivity: 75.2%	Specificity: 75.4%	
		+LR: 3.06	– LR: 0.33	

PPV: positive predictive value; NPV: negative predictive value;
+LR: positive likelihood ratio; – LR: negative likelihood ratio

Again, internal validation using bootstrapping suggests that the model is stable (
Table 2.18).

Table 2.18: Bootstrapping coefficients for combined gestational age / birthweight model for birthweights > 600g in NHS Scotland population dataset.

Covariate	**Level**	**Observed bootstrap coefficient (95% confidence interval)**	**P value**
Gestational age (weeks)		– 0.61 (--0.75- -0.48)	< 0.001
Birthweight		0.0008 (– 0.0001-0.001)	0.061
eFGR status	No	Reference	0.029
	Yes	0.64 (0.064-1.20)	
Constant		13.7 (10.7-16.6)	< 0.001

2.3.5. Prediction of neonatal death using NHS Scotland eFGR-specific data

2.3.5.1. Gestational age at delivery

In infants with a birthweight below the 3rd centile, each completed week's increase in gestational age at the time of delivery conferred a reduction in the risk of neonatal death following delivery of 45%. However, the relationship between gestational age and neonatal death is best modelled including gestational age as a quadratic term (P < 0.001; Model 2, Table 2.19). Inclusion of gestational age as a cubic term did not improve model performance, as confirmed by the non-significant model coefficients. These data are summarised by Table 2.19.

Table 2.19: Logistic regression model to predict neonatal death based on gestational age at delivery in eFGR using NHS Scotland population data (n = 926).

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 1			
Gestational age (weeks)	– 0.61 (– 0.74- – 0.48)	0.55 (0.48-0.62)	< 0.001
Constant	15.2 (11.5-18.9)		< 0.001
Model 2			
Gestational age (weeks)	– 7.16 (– 10.1-— 4.20)	0.0008 (0.000-0.015)	< 0.001
Gestational age^2 (weeks)	0.11 (0.06-0.17)	1.21 (1.07-1.18)	< 0.001
Constant	108 (65.7-151)	1	< 0.001
Model 3			
Gestational age (weeks)	– 41.9 (– 98.7-14.8)	$6.25e^{-19}$($1.43e^{-43}$-$2.74e^{5}$)	0.15
Gestational age^2 (weeks)	1.33 (– 0.65-3.30)	3.77 (0.52-27.1)	0.19
Gestational age^3 (weeks)	– 0.01 (– 0.04-0.009)	0.99 (0.96-1.01)	0.23
Constant	438 (– 103-980)		0.11

Likelihood ratio test:
Model 1 vs Model 2: P < 0.001
Model 2 vs Model 3: P = 0.12

2.3.5.2. Birthweight

In eFGR infants, an increase in birthweight of 25g confers a 12% reduction in the risk of neonatal death. The relationship between birthweight and neonatal death is however best modelled by including birthweight as a cubic term. These data are summarised by Table **2.**20.

Table 2.20: Logistic regression model to predict neonatal death based on birthweight in eFGR in NHS Scotland population data (n = 926).

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 1			
Birthweight (g)	– 0.13 (– 0.16-0.10)	0.88 (0.85-0.90)	< 0.001
Constant	2.32 (1.33-3.30)		< 0.001
Model 2			
Birthweight (g)	– 0.48 (– 0.64-0.33)	0.62 (0.53-0.72)	< 0.001
Birthweight^2	0.005 (0.003-0.008)	1.00 (1.00-1.00)	< 0.001
Constant	7.95 (5.29-10.6)		< 0.001
Model 3			
Birthweight (g)	– 1.57 (– 2.38- – 0.76)	0.21 (0.09-0.47)	< 0.001
Birthweight^2	0.03 (0.01-0.06)	1.04 (1.01-1.06)	0.002
Birthweight^3	$2.6e^{-4}$($4.5e^{-4}$-$6.9e^{-5}$)	0.99 (0.99-0.99)	0.007
Constant	20.0 (10.7-29.3)		< 0.001

Likelihood ratio test:
Model 1 vs Model 2: P < 0.001

Model 2 vs Model 3: P = 0.003

2.3.5.3. Gestational age and birthweight at delivery

Using both gestational age at delivery and birthweight to predict survival in a population of eFGR infants is best modelled using gestational age as a quadratic term (P = 0.003) and birthweight as a linear term (P = 0.01). There is a significant interaction between gestational age and birthweight (P = 0.02), indicating that the effect of birthweight on the likelihood of neonatal death is altered depending on the gestational age at delivery. These data are summarised In Table 2.21.

Table 2.21: Logistic regression model to predict neonatal death based on gestational age and birthweight in eFGR in NHS Scotland dataset (n = 926).

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Gestational age (weeks)	– 17.0 (– 0.57- – 0.07)	$4.18e^{-8}$($1.09e^{-12}$-$1.60e^{-3}$)	0.002
Gestational age^2 (weeks)	0.28 (0.10-0.45)	1.32 (1.10-1.58)	0.003
Birthweight	– 0.32 (– 0.57- – 0.07)	0.73 (0.56-0.93)	0.01
Birthweight* gestational age	0.02 (0.00-0.04)	1.02 (1.00-1.04)	0.02
Birthweight*gestational age^2	$3.22e^{-4}$($5.88e^{-4}$-$5.74e^{-5}$)	0.99 (0.99-0.99)	0.02
Constant	261 (107-417)		0.001

Test performance statistics (using a cut off of $\geq$ 0.05 to identify a predicted neonatal death) are summarised in Table 2.22.

Table 2.22: Test performance characteristics for combined gestational age / birthweight eFGR-specific model.

		Observed outcome		
		Neonatal death	*Survived to discharge*	
Predicted outcome	*Neonatal death*	43	125	PPV: 25.6%
	Survived to discharge	15	544	NPV: 97.3%
		Sensitivity: 74.1%	Specificity: 81.3%	
		+LR: 3.96	– LR: 0.32	

PPV: positive predictive value; NPV: negative predictive value;
+LR: positive likelihood ratio; – LR: negative likelihood ratio

Again, bootstrapping suggests stable performance of the model (Table 2.23).

Table 2.23: Bootstrapping coefficients for combined gestational age / birthweight eFGR-specific model.

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Gestational age (weeks)	– 16.0 (-28.3 –– 3.62)	$1.16e^{-7}$($1.15e^{-12}$- 0.01)	0.011
Gestational age^2 (weeks)	0.25 (0.04-0.46)	1.29 (1.06-1.57)	0.018
Birthweight	– 0.33 (– 0.62 - – 0.03)	0.72 (0.55-0.94)	0.029
Birthweight* gestational age	0.02 (0.001-0.04)	1.02 (1.00-1.03)	0.036
Birthweight*gestational age^2	– 0.0003 (– 0.0006- – 0.0001)	1.00 (1.00-1.00)	0.041
Constant	249 (67.6-432)		0.007

2.3.6. External validation of Scottish population models using St Mary's data

The St Mary's Hospital local dataset was used as a cohort for external validation of the models developed in the NHS Scotland data.

2.3.6.1. Combined gestational age / birthweight model

Applying these model coefficients to the St Mary's Hospital cohort and using the population based cut off of ≥ 0.05 to represent a neonatal death resulted in all cases in the St Mary's cohort predicted to end in a neonatal death.

2.3.6.2. Combined gestational age / birthweight model for cases > 600g

The test performance is summarised in Table 2.24. In this population, the model had an AUC of 0.68 (0.65-0.71).

Table 2.24: Test performance statistics for external validation of combined gestational age / birthweight for cases > 600g in St Mary's Hospital local dataset

		Observed outcome		
		Neonatal death	*Survived to discharge*	
Predicted outcome	*Neonatal death*	53	304	PPV: 14.8%
	Survived to discharge	14	612	NPV: 97.8%
		Sensitivity: 79.1%	Specificity: 66.8%	
		+LR: 2.38	– LR: 0.31 0	

PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; – LR: negative likelihood ratio

2.3.6.3. Combined gestational age / birthweight model for eFGR cases

Applying these model coefficients to the St Mary's Hospital cohort again resulted in all the eFGR cases predicted to be neonatal deaths.

2.3.6.4. Gestational age only model

Due to the failure of external validation of the combined models, the gestational age-only model was tested using the St Mary's Hospital cohort. In this cohort, it had an AUC of 0.75 (95% CI (0.72-0.78). The test performance is summarised in Table 2.25.

Table 2.25: Test performance statistics for external validation of gestational age only model in St Mary's Hospital local dataset.

		Observed outcome		
		Neonatal death	*Survived to discharge*	
Predicted outcome	*Neonatal death*	44	222	PPV: 16.6%
	Survived to discharge	23	694	NPV: 96.8%
		Sensitivity: 65.7%	Specificity: 75.8%	
		+LR: 2.71	– LR: 0.46	

PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; – LR: negative likelihood ratio

None of the population models here have been sufficiently validated using the St Mary's Hospital cohort as an external dataset to recommend their use in clinical practice. The next section of the results deals with using the St Mary's Hospital dataset only to develop a model for local use.

2.3.7. Prediction of neonatal death using St Mary's Hospital data

As this section of the analysis did not require a direct comparison with the NHS Scotland population data, births between 22^{+0} and 23^{+6} weeks' gestation were included. Given the small number of births at this gestation in the local cohort, individual case notes were able to be checked for accuracy.

2.3.7.1. *Gestational age at delivery*

The relationship between survival and gestation at delivery was investigated using logistic regression. It was found that this was best modelled including gestational age as a linear term, so the chance of neonatal death reduced as gestation increased (P < 0.001). The risk of death was higher in eFGR cases at any gestation, as shown by inclusion of eFGR status as a significant covariate (P < 0.001). The interaction between gestational age and eFGR status was non-significant (P = 0.29), suggesting that eFGR status does not influence how this relationship changes with advancing gestation. Regression coefficients for all models investigated are summarised in Table 2.26; model 4 (shaded grey) corresponds to the best fit model (gestational age as a linear term and eFGR status).

Table 2.26: Logistic regression model to predict neonatal death based on gestational age at delivery in births at St Mary's Hospital from 2012-2017 between 22-33 weeks' gestation.

Covariate	Level	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 1				
Gestational age (weeks)		– 0.34 (– 0.44-0.25)	0.71 (0.65-0.78)	< 0.001
Constant		7.41 (4.85-9.99)		< 0.001
Model 2				
Gestational age (weeks)		1.13 (– 0.99-3.25)	3.10 (0.37-25.8)	0.30
Gestational age^2 (weeks)		– 0.03 (– 0.06-0.01)	0.97 (0.94-1.01)	0.17
Constant		– 13.4 (– 43.4-16.6)		0.38
Model 3				
Gestational age (weeks)		13.2 (– 22.7-49.0)	13.15 (– 22.7- 49.0)	0.47
Gestational age^2 (weeks)		– 0.45 (– 1.70-0.81)	0.63 (0.18-2.25)	0.49
Gestational age^3 (weeks)		0.005 (– 0.01-0.02)	1.00 (0.99-1.02)	0.51
Constant		126 (– 467-213)		0.46
Model 4				
Gestational age (weeks)		– 0.40 (– 0.50- – 0.29)	0.67 (0.61-0.75)	< 0.001
eFGR status	No	Reference	Reference	
	Yes	1.42 (0.85-2.00)	4.16 (2.33-7.40)	< 0.001
Constant		8.49 (5.66-11.3)		< 0.001
Model 5				
Gestational age (weeks)		– 0.07 (– 0.08- – 0.05)	0.93 (0.92-0.95)	< 0.001
eFGR status	No	Reference	Reference	
	Yes	– 1.84 (– 7.98-4.30)	0.16 (0.00-74.0)	0.56
eFGR status*gestational age		0.02 (– 0.01-0.05)	1.02 (0.99-1.05)	0.29
Constant		10.6 (7.96-13.1)		< 0.001

Likelihood ratio test:
Model 1 vs Model 2: P = 0.47
Model 2 vs Model 3: P = 0.009
Model 1 vs Model 4: P < 0.001
Model 4 vs Model 5: P = 0.30

Figure 2.10 shows how the relationship between gestational age and predicted probability of neonatal death changes, and how eFGR status influences this.

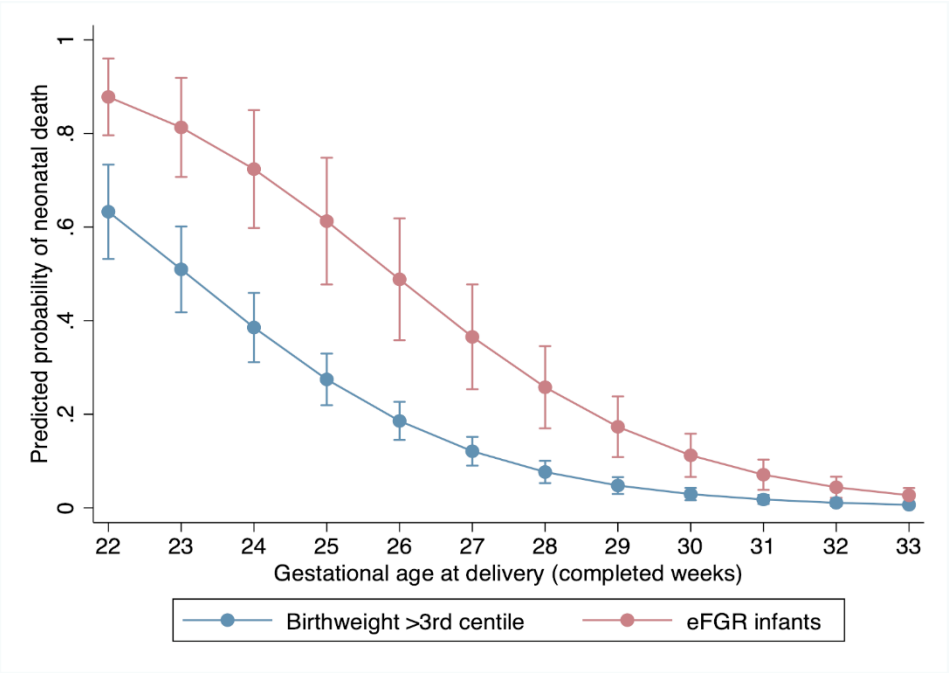


Figure 2.10: Predicted probability of neonatal death according to gestational age at delivery for non-eFGR (blue) and eFGR (red) infants from 22-33 weeks' gestation. Based on logistic regression model using St Mary's Hospital data.

The probability of death falls for both eFGR and non-eFGR cases across the gestational ages studied. There is a significant difference between eFGR and non-eFGR cases, with eFGR cases having a much higher predicted probability of neonatal death, until 32 weeks' gestation when this becomes similar to non-eFGR cases.

2.3.7.2. Birthweight

The relationship between birthweight and neonatal death was best modelled using birthweight as a quadratic term Table 2.27. The risk of neonatal death falls as birthweight increases ($P < 0.001$) for all cases. Inclusion of eFGR status as either a covariate or an interaction term does not have a significant impact on prediction, suggesting that, in this cohort of infants, the relationship remains the same in eFGR and non-eFGR pregnancies.

Table 2.27: Logistic regression model to predict neonatal death based on birthweight (25g increments) in births at St Mary's Hospital from 2012-2017 between 22-33 weeks' gestation.

Covariate	Level	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 1				
Birthweight		– 0.09 (– 0.11- – 0.07)	0.92 (0.90-0.93)	< 0.001
Constant		1.32 (0.68-1.97)		< 0.001
Model 2				
Birthweight		– 0.21 (– 0.27- – 0.15)	0.81 (0.77-0.86)	< 0.001
Birthweight^2		0.001 (0.000-0.002)	1.00 (1.00-1.00)	< 0.001
Constant		3.55 (2.31-4.79)		< 0.001
Model 3				
Birthweight		–0.37 (–0.58—0.16)	0.69 (0.56-0.85)	< 0.001
Birthweight^2		0.005 (0.00-0.01)	1.00 (1.00-1.01)	0.04
Birthweight^3		–2.29e^{-5}(–5.3e^{-5}-7.25e^{-6})	1.00 (0.99-1.00)	0.14
Constant		5.64 (2.74-8.55)		< 0.001
Model 4				
Birthweight		–0.21 (–0.27- –0.15)	0.81 (0.77-0.86)	< 0.001
Birthweight^2		0.001 (0.001-0.002)	1.00 (1.00-1.00)	< 0.001
eFGR status	No	Reference	Reference	
	Yes	–0.10 (–0.65-0.44)	0.90 (0.52-1.56)	0.71
Constant		3.56 (2.32-4.83)		< 0.001
Model 5				
Birthweight		–0.21 (–0.28- -0.14)	0.81 (0.76-0.87)	< 0.001
Birthweight^2		0.001 (0.001-0.002)	1.00 (1.00-1.00)	< 0.001
eFGR status	No	Reference	Reference	
	Yes	2.23 (–1.99-6.45)	9.29 (0.14-631)	0.3
eFGR status*birthweight		–0.16 (–0.40-0.08)	0.85 (0.67-1.09)	0.2
eFGR status*birthweight^2		0.002 (–0.001-0.005)	1.00 (0.99-1.01)	0.1
Constant		3.62 (2.18-5.06)		< 0.001

Figure 2.11 shows the change in the predicted probability of neonatal death as birthweight increases. Data are not split according to eFGR status here as it did not have a significant impact on the relationship between survival and birthweight.

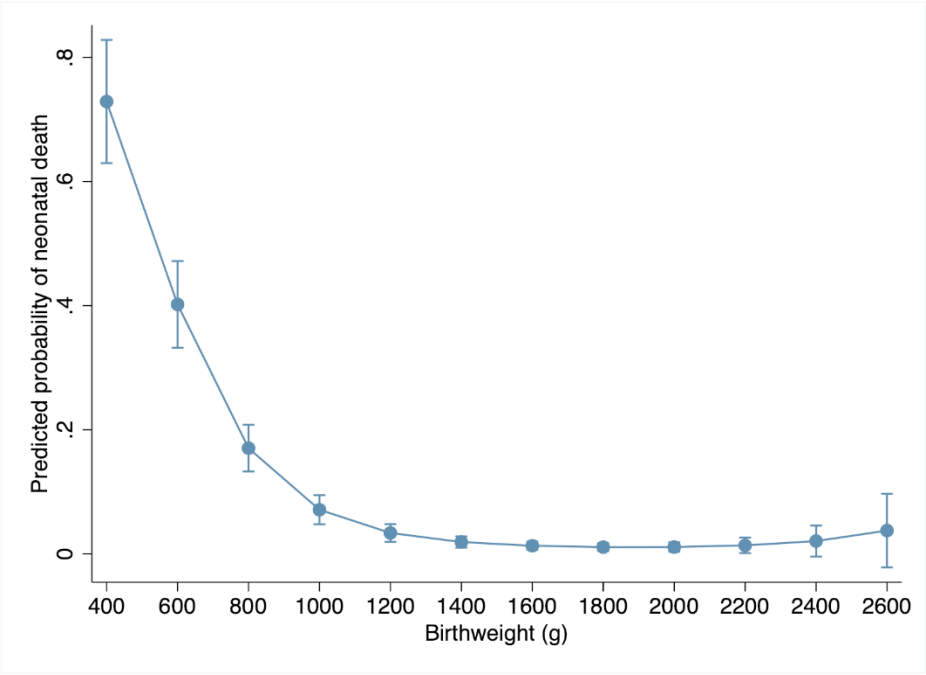


Figure 2.11: Predicted probability of neonatal death according to birthweight for infants born between 22-33 weeks' gestation. Based on logistic regression model using St Mary's Hospital data from 2012-2017.

2.3.7.3. Gestational age and birthweight at delivery

Gestational age at delivery and birthweight can also be considered together to predict survival chances. This is best modelled using both gestational age (P = 0.001) and birthweight (P < 0.001) as linear terms (Table 2.28). There is a significant interaction between gestational age and birthweight (P = 0.003), therefore indicating that the effect of birthweight on the likelihood of neonatal death is altered depending on the gestational age at delivery. eFGR status does not impact on survival chances, when considered either as an independent covariate (P = 0.61) or an interaction term (P = 0.32).

Table 2.28: Logistic regression model to predict neonatal death based on gestational age at delivery and birthweight (25g increments) in births at St Mary's Hospital from 2012-2017 between 22-33 weeks' gestation.

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
Model 11			
Gestational age (weeks)	–0.30 (–0.48- –0.11)	0.74 (0.62-0.89)	0.001
Birthweight	–0.32 (–0.50- –0.15)	0.73 (0.61-0.86)	< 0.001
Birthweight*gestational age	0.008 (0.003-0.01)	1.01 (1.00-1.01)	0.003
Constant	9.61 (4.58-14.6)		< 0.001

Combining gestational age at delivery and birthweight provides a more accurate prediction of survival chances than using either variable alone, as proven by a significant likelihood ratio test (P < 0.001). This model has an AUC of 0.87 (95% CI (0.83-0.90). The test performance is specified in Table 2.29. A cut off value of 0.1 was used to denote a positive event (neonatal death). Internal validation using bootstrapping suggested that the model was stable in this dataset (Table 2.30).

Table 2.29: Test performance statistics for combined gestational age / birthweight model in St Mary's dataset.

		Observed outcome		
		Neonatal death	*Survived to discharge*	
Predicted outcome	*Neonatal death*	101	229	PPV: 30.6%
	Survived to discharge	31	988	NPV: 96.7%
		Sensitivity: 76.6%	Specificity: 81.2%	
		+LR: 4.07	–LR: 0.29	

PPV: positive predictive value; NPV: negative predictive value;
+LR: positive likelihood ratio; –LR: negative likelihood ratio

Table 2.30: Bootstrap coefficients for combined gestational age / birthweight model (500 replications).

Covariate	Observed bootstrap coefficient (95% confidence interval)	P value
Gestational age (weeks)	–0.43 (–0.62- –0.23)	< 0.001
Birthweight	–0.016 (–0.02-–0.008)	< 0.001
Gestational age*birthweight	0.0004 (0.0001-0.0006)	< 0.001
Constant	13.4 (8.02-18.7)	< 0.001

This model has subsequently been used to create a survival chart, which can be used to calculate the estimated chance of survival with a 95% confidence interval, depending on birthweight and gestational age at delivery; this is included as Figure 2.12. The estimated model is shown below:

$$Log\ odds\ of\ survival\ to\ discharge = (0.30 \times gestational\ age\ (completed\ weeks)) + 0.32 \times birthweight\ (g) + (0.0008 \times birthweight \times gestational\ age) - 9.61.$$

This chart can be used to counsel parents about the likelihood of survival once a decision is made regarding delivery, for both eFGR and non-eFGR cases. For example, if an infant is born at a weight between 600-800g at 26 weeks, its estimated chance of survival is 68%.

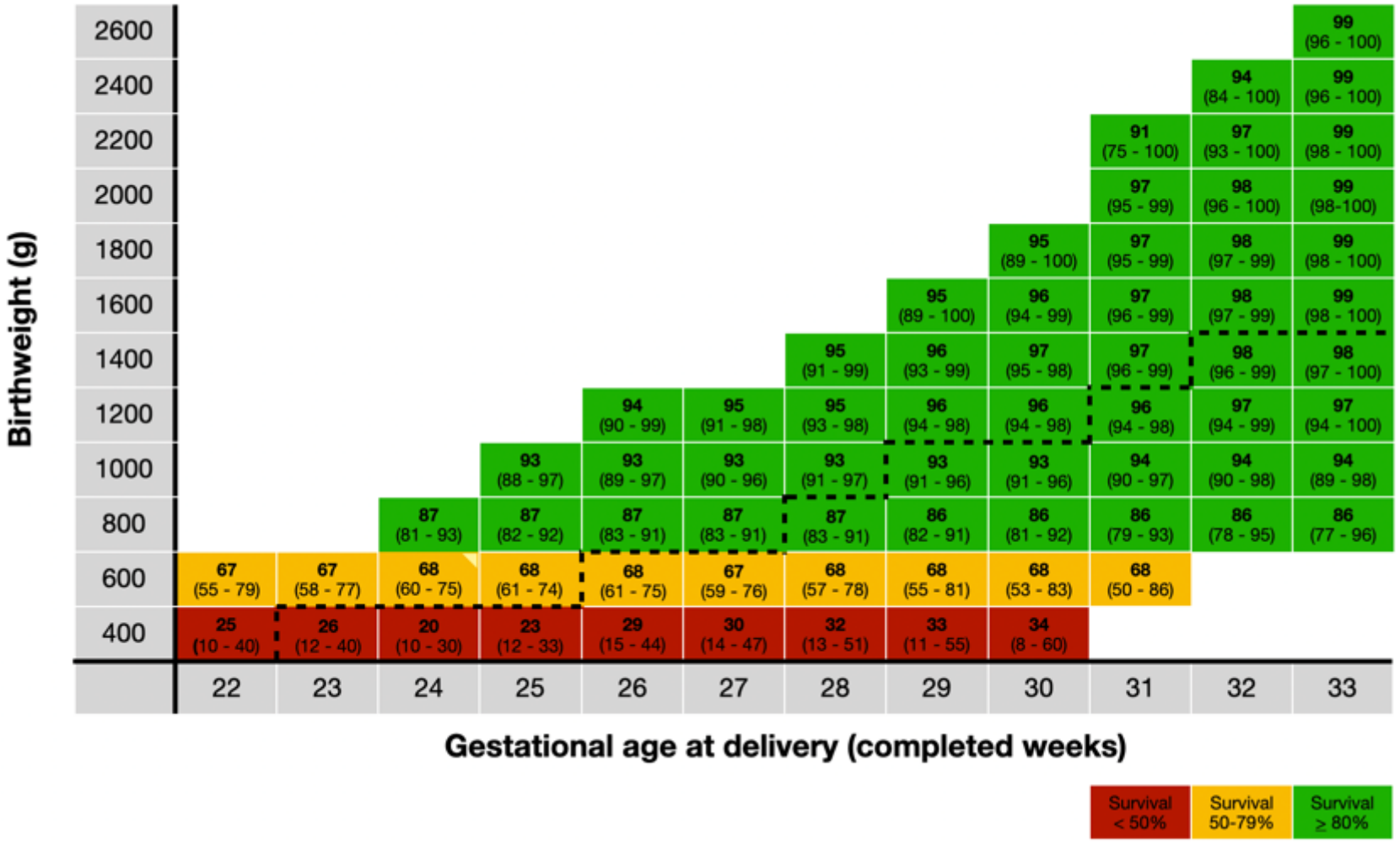


Figure 2.12*:* Probability of survival to hospital discharge (95% confidence interval) following live birth based on gestational age at delivery and birthweight. Below the dashed line represents eFGR (birthweights below the 3rd centile). Each group is colour coded according to survival (see legend).

2.4. Discussion

The work presented in this chapter confirms the overall incidence of eFGR to be approximately 3 per 1000 births in a population dataset. In a large tertiary unit, it is slightly higher at 5 per 1000 births, reflective of the higher complex case load at such a unit.

Analysis of population data relating to singleton non-anomalous births between 24 and 33 completed weeks' gestation from 2012 to 2017 in Scotland has confirmed that birthweight and gestational age are predictive of neonatal death, and that eFGR status impacts on that relationship. Logistic regression has been used to attempt to create a model from this data to predict the likelihood of neonatal death based on gestational age at birth and birthweight and eFGR status. Regression diagnostics suggest a poor fit of the model to the data, and external validation of the model using a matched data set from St Mary's Hospital failed to predict neonatal death. The local St Mary's dataset has also been used to explore the relationship between gestation at delivery, birthweight, eFGR status and neonatal death. The resulting model has been used to create a survival chart which can be used by clinicians to guide the delivery decision-making and provide improved counselling about potential outcomes to affected parents.

2.4.1. Strengths and limitations

The major strengths of this study are that the data are obtained from a large national population database, with reliable birth records. This database has previously been used for large scale population studies (179,180). Obstetric practice across Scotland is likely representative of other western societies, and therefore the conclusions drawn here can be applied widely. The local dataset has been taken from a tertiary unit in the UK. The relatively small number of cases involved has meant that data can be cross-checked across multiple platforms to ensure accuracy.

This study does have a number of limitations. The birth data includes gestational age in completed weeks only. At these early gestations, days can have an impact on pregnancy outcome; therefore this level of detail would be valuable in predicting the chance of a pregnancy ending in a live birth or an infant surviving the neonatal period. Defining survival based on completed weeks of gestation assumes that a baby born at 24^{+0} behaves the same as a baby born at 24^{+6} weeks' gestation, and both the work in this chapter and previous work have shown that survival chances change greatly within the space of just one week at these early gestations (64,70,73,174,181). In the same way, this method of data collection also obscures the similarities between a baby born at 24^{+6} and 25^{+0} and will assign a different survival chance to both of them. The definition of eFGR involves the use of umbilical artery Doppler status, which is not provided through the Scottish database. This means that the definition of eFGR used in this study had to be modified, so only those cases with a birthweight <3rd centile were classed as eFGR. It would not be routinely expected, however, that this level of detailed data would be available in such a large-scale population dataset. Although the generalisability to other developed countries was previously mentioned as a strength of this study, it could be argued that due to the ethnic profile of the population studied, findings may not necessarily be applied across all developed nations. Finally, in the case of iatrogenic delivery, the

gestational age and therefore the birthweight at delivery are hugely influenced by clinician behaviour. The majority of eFGR cases included in the dataset will have been planned deliveries, rather than spontaneous births. Therefore, the relationship between gestation, birthweight and survival is greatly influenced by clinical decision-making around the decision to deliver, and this is not considered through this analysis. To obtain a truer picture of survival, only spontaneous births should be included, but this is not a feasible method of investigating survival in eFGR, as without intervention most cases would end in FDIU.

2.4.2 Incidence

The data reported here represent the first study to determine the incidence of eFGR in the general obstetric population. This confirms that it is an uncommon condition, so is best managed in a tertiary unit by a specialist maternal and fetal medicine team, who have access to level 3 NICU care. This approach has several advantages. Firstly, care given by a dedicated team would ensure uniform management across a region; management can vary greatly between hospitals and obstetricians, particularly between tertiary units and district general hospitals, and this is undoubtedly influenced by the level of experience of the managing clinician (182). Anecdotally, it seems that district general hospitals have a lower threshold for delivery (183), with cases delivered at an earlier gestation and a lesser degree of compromise than in tertiary units, where those with more experience probably have the confidence to continue the pregnancy further to gain gestation and birthweight. This, as the work in this chapter confirms, is likely to have a big impact on infant survival. From a patient perspective, management in a tertiary unit with level 3 neonatal care would ensure continuity of care across the antenatal, delivery and postnatal periods, which may offer some psychological support through an undoubtedly difficult emotional time.

2.4.3. Comparison of the two populations

Comparing the Scottish data with the St Mary's data shows some differences between the two populations. The incidence of eFGR in St Mary's is higher than across the whole of Scotland (4.9 compared to 2.8 per 1000 births), which reflects St Mary's status as a tertiary unit with a specialist fetal medicine unit and a dedicated fetal growth restriction clinic, and the high-risk population that it serves. There appears to be more fluctuation in the rate over the study period in the St Mary's data compared to Scotland. The apparent spike in 2015 to 6 per 1000 pregnancies was likely due to the STRIDER study, which aimed to test sildenafil as a treatment to prolong gestation in cases of eFGR. St Mary's was the lead recruiting site (15).

It is difficult to make firm statistical comparisons between the two populations because ethical constraints of the National Safe Haven, within which the Scottish dataset was accessed and analysed, prevented the St Mary's dataset being uploaded for direct comparison. The following discussion relates to likely trends, not confirmed differences noted within the data.

In terms of population demographics, the most striking differences between the two datasets are in the ethnic diversity, with St Mary's having a significantly lower proportion of white British females than Scotland (57.7% compared to 70.6%) and a higher proportion of minority ethnic groups. The St Mary's dataset would suggest there is a difference between those women that develop eFGR

and those that do not in terms of ethnicity, with minority ethnic groups having a 33% increased risk of eFGR, and only 50% of eFGR cases occurring in those classified as white, compared to 68% in Scotland. Almost a quarter of eFGR cases in the Scottish population were classified as other / unknown ethnicity, but the proportion in the St Mary's dataset was only 8.5%, therefore even if it is assumed that all the unknown cases in the Scottish data were from a minority ethnic background, this discrepancy would not alter the fact that a much lower proportion of cases in the smaller St Mary's dataset are classified as white. This is not, however, seen in the much larger Scottish population data. 2011 census data from Scotland and England and Wales confirms that the percentage of people from minority ethnic groups was only 4% in Scotland (184) compared to 14% across England and Wales (185), therefore these differences in ethnicity are not unexpected.

The Scottish dataset suggests that there is a significant difference in maternal height between those pregnancies that develop eFGR and those that do not, which may be related to unmeasured confounding factors such as social deprivation or ethnicity. The difference however is small and unlikely to be of clinical significance. In both groups, cases of eFGR were more likely to occur in primiparous than multiparous women; this is in keeping with nulliparity as a known risk factor for FGR (186). There was a similar pattern in the distribution of infant sex in both datasets, with eFGR infants having a higher proportion of females. This finding mirrors previous work which has identified that female fetuses are more likely to be affected by FGR (187). Maternal conditions were recorded in the NHS Scotland population dataset. eFGR cases were associated with higher rates of pre-eclampsia, which is to be expected given that they both reflect placental dysfunction and frequently co-exist (9). eFGR was also associated with a higher incidence of pre-existing hypertension and chronic kidney disease, which again are associated with a higher risk of placental dysfunction (188). Data regarding previous perinatal morbidity and mortality were not available for the NHS Scotland data, but from the St Mary's data it is noted that those patients who had had a previous FDIU were more likely to have an FDIU again. This is not surprising given that previous FGR is a risk factor for FGR (189), and pathologies such as MVM and CHI, which are implicated in eFGR, often reoccur (39,190).

The biggest difference in pregnancy outcomes in eFGR was in the live births and neonatal death rates; Scotland had a lower neonatal death rate within the eFGR cohort than was found in the St Mary's population. This is likely to reflect St Mary's status as a tertiary referral unit, meaning it is likely to care for the most complicated of cases with a higher risk of death. In the Scottish eFGR cohort, almost a three-quarters of infants (72.5%) were delivered via Caesarean section, compared to two-thirds (65.2%) in the St Mary's eFGR cohort. This difference is difficult to account for without knowing more details about each case. Vaginal delivery could be used as a proxy for spontaneous birth, as planned delivery of eFGR infants is recommended via Caesarean section due to the compromised nature of these very small, very early fetuses and the fetal distress that labour would undoubtedly cause (191,192). In that case, it would be expected that St Mary's would have a lower proportion of spontaneous deliveries due to the higher incidence of eFGR and the high proportion of referrals it receives from neighbouring units which are more likely to be delivered via planned intervention, but this was not the case here. This could be further investigated by splitting the

Scotland population data according to the type of hospital where the delivery took place, but this level of detail was not available.

2.4.4. Predicting survival by gestational age and birthweight

This study has confirmed that both gestational age and birthweight are predictors of survival and has added further information regarding the relationship between gestation, birthweight and survival specific to eFGR. In terms of gestational age, the probability of neonatal death falls as gestational age increases, but the population level data show that eFGR infants behave differently to their non-eFGR counterparts. The risk of neonatal death for eFGR infants is much higher, as would be expected, but beyond 28 weeks' gestation there appears to be little difference between the risk of death in eFGR and non-eFGR cases. This analysis does not address the question regarding long-term morbidity in eFGR however. Local data collected from St Mary's Hospital again confirm that eFGR infants have a higher likelihood of neonatal death, but these data do not suggest the same relationship between gestational age, eFGR status and neonatal death. Locally, eFGR and non-eFGR cases do not seem to reach a similar level of risk of death until 32 weeks' gestation. Obviously, this is based on much smaller numbers, but could be reflective of differences in the populations and also the type of cases that St Mary's Hospital typically cares for which tend to be more complex than the general population.

eFGR status also impacts on the relationship between birthweight and neonatal death, with survival typically higher in eFGR infants born at a specific weight compared to a non-eFGR infant born at the same weight. An eFGR infant born at 1000g, for example, would typically be born at around 30 weeks' gestation, whereas an appropriately grown 1000g infant with a birthweight on the 50th centile would be born around 27 weeks' gestation. This could therefore reflect the survival advantage associated with increasing gestational age. The model developed using the St Mary's Hospital data suggested a different relationship between survival and gestational age, birthweight and eFGR status, however this may be related to the smaller sample size of this population, meaning that significance is not reached.

Unfortunately, this work was not able to create a clinically useable population-based model to predict survival based on gestational age at delivery and birthweight. One of the original objectives of this work was to improve antenatal counselling of parents by having this information to offer at the time of discussion about prognosis and the decision to deliver. Exploration of alternative models using only cases with a birthweight above 600g suggested that the relationship between gestation, birthweight and survival is different below 600g, or there are inaccuracies in the data recorded for those births at the lowest birthweights / earliest gestations. On the other hand, the St Mary's dataset may not be a suitable choice for external validation, given the differences highlighted previously in this discussion.

A model combining both gestation and birthweight was developed using the St Mary's Hospital data: eFGR status was not found to be significant either as a covariate or an interaction factor, but there was a significant interaction between birthweight and gestation. The interplay between

gestational age at delivery, birthweight and survival is complex. In cases of eFGR when the delivery is planned rather than spontaneous, the gestational age at delivery is heavily influenced by the obstetrician, who will choose the point at which to deliver. Beyond the point of reaching a weight considered viable, the birthweight at delivery cannot be controlled to the same extent. The population data presented in this chapter suggest that in terms of gestation, beyond 28 weeks there is little survival advantage to be gained by prolonging the pregnancy. When considering birthweight in isolation, the same point is reached at approximately 1200g, which the majority of eFGR infants will not reach by definition because this would be reached at a gestational age of 32 weeks or beyond. When considering the survival chart which summarises the St Mary's combined model of gestation and birthweight, it can be seen that advancing gestation whilst birthweight remains constant provides little survival advantage. For example, if a baby is born weighing between 600-800g at 26 weeks, its survival chance is 67% (95% CI 61-75%), and this remains the same at 27 weeks (69% survival chance (95% CI 59-76%). This suggests that in the event of static growth, which is frequently seen in eFGR, birthweight is the bigger predictor of survival, and prolonging gestation (which is the only active management that can be offered) confers little survival advantage. In addition, prolonging gestation brings with it the added risk of FDIU, which was not considered here.

2.4.5. Future work

Although this chapter has allowed a survival chart specific to the St Mary's Hospital population to be developed, a similar population level chart using the Scottish data could not be developed. Future work in this area should focus on determining if this is possible, and if so, what the most suitable population for model development would be. This discussion has previously alluded to the differences between care in tertiary and district general level hospitals. Given the confirmed low incidence of eFGR and the need for it to be managed in a tertiary-level unit, it may be more appropriate for the model to be developed using data from tertiary-level units only. However, as this is such a rare condition, to obtain adequate numbers for analysis, it may need to be extended outside of the UK. One such population database to explore would be the Swedish Medical Birth Register, which is a well-established, accurate health data register that was begun in 1973 (193). Following from development of a successful model, further comparisons could be made to consider infant sex and maternal ethnicity.

This work has provided an initial exploration of the relationship between gestational age, birthweight and survival by providing a basic understanding based on parameters at birth. To provide a more accurate estimation of survival, it needs to consider the ongoing risk of FDIU as the pregnancy progresses. This could be approached using a competing risks model, which would allow pregnancies ending in both FDIU and neonatal death to be included.

2.4.6. Conclusion

This is the first study to confirm the incidence of eFGR on a population level, at 3 per 1000 births. It attempted to explore the relationship between gestational age at delivery and birthweight with neonatal death in both a nationwide population of pre-33 week births and a local cohort at a tertiary-level unit, with a focus on eFGR. Both gestational age and birthweight are significantly

inversely associated with the chance of neonatal death. Important considerations should be made within the context of eFGR, however. Survival at a given gestation is higher in non-eFGR cases, until 28 weeks' gestation, where there is little difference between the two groups. In terms of birthweight, an eFGR infant born at a birthweight below 1200g is more likely to survive than its appropriately grown counterpart, as a reflection of the later gestation at which birth occurs. Combining gestation and birthweight to create a predictive model that considers both variables was not feasible in the population studied; this may reflect the population level data, or the local cohort that was used to validate the data, given that there were differences between the two. At a local level, a model was successfully developed for use in the local population and suggested that there was little survival advantage to be gained by increasing gestation at a given birthweight, with the main difference in survival coming from increasing birthweight at a given gestation. This implies that in the scenario of static growth in eFGR, there is little advantage to be gained in advanced gestation. It is important to note that the data here also deals with neonatal death and survival to discharge (within one year) as a binary outcome. For affected parents, however, the pregnancy outcome is much more than a simple death vs. survival prediction, with eFGR and preterm birth associated with high rates of morbidity and neurodevelopmental problems extending far beyond the neonatal period, which this study has not addressed. It is also important to remember that the data presented here represent the estimated risk based on population averages and do not provide the estimated risk of an outcome for a given individual. Individual risk prediction in the context of eFGR will be explored further in the following chapter.

CHAPTER 3: IDENTIFICATION OF PROGNOSTIC FACTORS IN eFGR

3.1. Introduction

eFGR is diagnosed on the basis of ultrasound findings, yet there has been no work to date to identify which, if any, ultrasound findings are indicative of prognosis at the time of diagnosis. Findings such as UA absent EDF or oligohydramnios are widely believed to be poor prognostic indicators (11), but the relationship between these factors and pregnancy outcome has never been quantified in eFGR. The previous chapter has improved current understanding of survival in eFGR based on gestational age at delivery and birthweight, but this was largely based on population level data. This work presented in this chapter aims to better characterise the course of eFGR, and also provide prognostic information on an individual level. Women with eFGR undergo multiple ultrasound scans throughout the course of their pregnancy, and this therefore represents a huge body of data which can be explored to better characterise the course of eFGR, and also determine how scan features can be predictive of prognosis. In addition to using the scan at the time of diagnosis to predict prognosis, longitudinal change in factors such as fetal weight gain and maternal and fetal Doppler progression can be investigated to assess how these also relate to pregnancy outcome. If predictive factors are identified, then combining these into a predictive model would create a clinically useful tool that can guide clinicians with their decision making, if and when to intervene in eFGR, and better inform affected families regarding the likely outcome of the pregnancy.

3.2. Methods

Ethical approval was obtained from the Health Research Authority and West Midlands Research Ethics Committee (IRAS ID: 248646; REC reference: 19/WM/0023), and all work was conducted in accordance with the Declaration of Helsinki 1975 (revised 2013). As the study involved a secondary use of retrospective, routinely collected patient data, individual written patient consent was not required for data access. Patient identifiable information was only accessed by members of the clinical care team during the initial search, and all data retained for analysis were pseudo anonymised, therefore an application to the Confidential Advisory Group was not necessary. Hospital trusts included on the HRA/ethics application included Manchester University NHS Foundation Trust (St Mary's Hospital, Oxford Road campus; primary site) and East Lancashire Hospitals NHS Trust (Burnley General Teaching Hospital). For the purposes of this thesis, the main database contains only data collected from St Mary's Hospital, Manchester. One section of the results concerning longitudinal weight change (Section 3.2.7.1) combines data from St Mary's Hospital with data from the Placenta Clinic at Burnley General Teaching Hospital.

3.2.1. Definition

To define eFGR, the Delphi consensus definition of FGR of AC < 3rd centile, EFW < 3rd centile and absent umbilical artery EDF, or AC/EFW <10th centile with umbilical artery or uterine artery PI > 95th centile was used (8), with an additional gestational age caveat of pre-28^{+0} weeks' gestation for diagnosis and pre-33^{+0} weeks' gestation for delivery.

3.2.2. Data collection/search

The Viewpoint Version 5 radiology reporting system (GE Healthcare, Chalfont St Giles, UK) was used to identify those women with a diagnosis of eFGR who were reviewed in the translational research antenatal clinics from April 2009 – July 2019. The search strategy used is outlined in Figure 3.1.

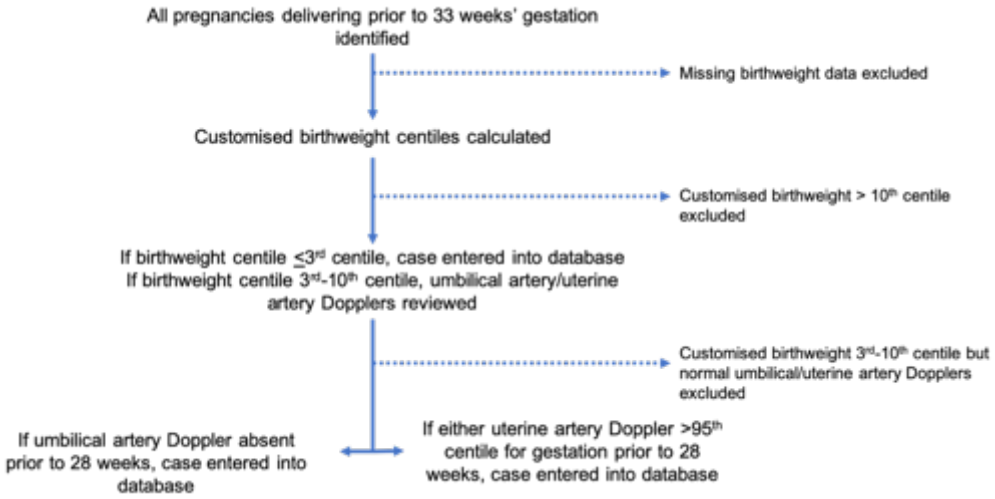


Figure 3.1: Search strategy used to identify eligible eFGR cases using Viewpoint 5.

To ensure that no cases were missed due to missing pregnancy outcome data, a further search was also performed to identify those cases with absent UA EDF prior to 28^{+0} weeks' gestation, and those scans when a ductus venosus Doppler was performed (as this would usually only be performed in cases of early-onset FGR). This identified a further 45 cases, which had been missed in the initial search due to missing pregnancy outcome data. Missing outcome data were subsequently obtained by searching the St Mary's Hospital IT systems or contacting the hospital where the birth took place. Unfortunately, pregnancy outcome data remained missing in 18 cases (three of which were known to have ended in FDIU, which had been confirmed on scan at St Mary's Hospital, but delivery occurred elsewhere), where the hospital responsible for delivery could not be determined, or delivery was recorded but without any birthweight data.

Following identification of cases that met the inclusion criteria, booking data (including maternal characteristics and obstetric history), pregnancy outcome data and data from all ultrasound scans (including both growth and Doppler only scans) performed during that pregnancy were obtained from Viewpoint. Individual variables collected are summarised in Table 3.1. Scans were sequentially numbered by date and labelled as either growth or Doppler only scans. These data formed the master database, from which all subsequent analysis was performed.
Neonatal outcome data were collected where available using the BadgerNet Neonatal Electronic Patient Record Version 2.9.1.0 (Clevermed, Edinburgh, UK). Data collected for each eFGR case born alive is summarised by Table 3.1. Only notes recorded at St Mary's Hospital, Manchester could be directly accessed so only clinical data from this hospital stay could be recorded, however data regarding the length of stay at other units could be accessed through the BadgerNet system.

Table 3.1: List of variables collected from Viewpoint 5 / BadgerNet for each eFGR case identified.

Maternal characteristics	Age (years) Booking weight (kg) Booking height (cm) Body mass index (BMI) kg/m^2 (calculated from maternal booking data) Booking systolic blood pressure (mmHg) Booking diastolic blood pressure (mmHg) Smoking status Alcohol intake Ethnicity
Obstetric history	Number of previous live births $\geq 37^{+0}$ weeks Number of previous live births $< 37^{+0}$ weeks Number of previous neonatal deaths $\geq 37^{+0}$ weeks Number of previous neonatal deaths $< 37^{+0}$ weeks Number of previous miscarriages $< 16^{+0}$ weeks Number of previous miscarriages 16^{+0}-22^{+6} weeks Number of previous FDIUs $\geq 37^{+0}$ weeks Number of previous FDIUs $< 37^{+0}$ weeks Gravida and parity
Ultrasound parameters (recorded at each scan episode)	Gestational age (weeks + days) at scan (based on routine first trimester dating) Scan date **Fetal biometry** Biparietal diameter (BPD) (mm) Head circumference (HC) (mm) Abdominal circumference (AC) (mm) Femur length (FL) (mm) Estimated fetal weight (EFW) (g) **Liquor volume** Single deepest vertical pool (cm) Amniotic fluid index (cm) **Fetal Dopplers** Umbilical artery (UA) – pulsatility index (PI)/resistance index (RI)/end-diastolic flow (EDF) Middle cerebral artery (MCA) – PI/RI/V_{max} Ductus venosus – pulsatility index for veins (PIV)/PVIV/a-wave **Maternal Dopplers** Uterine artery (UtA; both right and left) – PI/RI/presence of notch **Placental biometry** Placental diameter (cm) x 2 Placental depth (cm) Placental efficiency coefficient (PEC; (placental diameter*placental diameter)/placental width) Placental surface area (PSA; placental diameter*placental diameter) **Other** Systolic blood pressure (mmHg) Diastolic blood pressure (mmHg)
Pregnancy outcome	Gestational age at delivery (weeks + days) Birthweight (g) Infant sex Mode of delivery Outcome – live birth discharged from hospital/FDIU/neonatal or infant death
Neonatal complications	Length of neonatal stay Destination at discharge (home/another hospital) Presence of necrotising enterocolitis/chronic lung disease/retinopathy of prematurity/confirmed sepsis/intraventricular haemorrhage or periventricular leukomalacia

All statistical analysis was performed using Stata 15.1 for Mac (StataCorp, College Station, TX, USA), or R: A language and environment for statistical computing (176); figures were produced using Stata 15.1 for Mac or the graphical package ggplot2 (194).

3.2.3. Characterisation of cohort

Maternal booking data and pregnancy outcome data were used to characterise the eFGR cohort. For the continuous variables, normality was determined through plotting a population density curve and using the Shapiro-Wilk normality test. Mean ± standard deviation was calculated for parametric data, or median (range) for non-parametric data as appropriate. For categorical variables, data are presented as counts (percentage).

For comparison, data were split according to outcome (live birth discharged from hospital/FDIU/neonatal (during first 28 days of life) death (NND) or infant (up to one year of life) death). Continuous variables were compared between the three groups using ANOVA with Tukey's post hoc test for parametric data, or Kruskal-Wallis for non-parametric data, as appropriate. Chi-squared test was used to compare categorical variables across the three groups. For all comparisons, a P value <0.05 was considered statistically significant.

To determine baseline ultrasound characteristics of the cohort, ultrasound parameters measured at the first recorded scan in the translational research clinics were used – this scan was taken as the "diagnosis scan". Analysis was repeated using only those cases where the first scan was performed between 21^{+0}-23^{+6} weeks' gestation (the gestation at which a "placental screen" is undertaken in St Mary's Hospital). Continuous and categorical data were presented and compared as described above.

3.2.4. Neonatal outcome data

Data were again split according to outcome – survival to discharge compared with neonatal/infant death. For continuous variables (neonatal length of stay (LoS)), normality was determined using the Shapiro-Wilk normality test, then data presented as mean ± standard deviation or median (range) as appropriate and compared using t-test/Mann-Whitney U-test respectively. For categorical variables, data are presented as counts (percentage) and compared between groups using Chi-squared tests or Fisher's exact test (for counts of less than five). For all comparisons, a P value < 0.05 was taken as statistically significant.

3.2.5. Identifying prognostic factors

The PROGnosis RESearch Strategy (PROGRESS), which outlines a framework for prognosis research, was adhered to where possible for this section of the analysis (195,196).

Logistic regression was used to investigate the relationship between maternal characteristics and ultrasound parameters at the placental screen, and pregnancy outcome. Pregnancy outcome was characterised in two different ways for the purposes of analysis:

1) FDIU compared to live birth;
2) overall death (composite of FDIU and neonatal and infant death) compared to survival to discharge.

Univariable analysis was performed using a generalised linear model assuming a binomial family with logistic link. To account for the differences in gestational age at the time of the first recorded scan, gestational age (days) was included as a covariate to allow the relationship to be adjusted for gestation. Results are presented as odds ratios with 95% CI. P values are reported, with a P value of < 0.05 taken to be statistically significant.

3.2.5.1. Multivariable model

Following univariable analysis, those variables deemed to be prognostic of pregnancy outcome were taken forward to be used in multivariable logistic regression, to develop a predictive model which could be used in clinical practice to predict pregnancy outcome at the time of diagnosis of eFGR. The significance level cut off of $P < 0.05$ was not applied for variable selection, as it was felt that variables approaching significance could potentially improve the model performance when selected in conjunction with other variables, so a more liberal value of $P < 0.2$ was chosen for retention. Multivariable analysis was performed in a backwards stepwise process, with all candidate variables initially included, then variables removed sequentially to find the combination with the best predictive ability. Predictive ability was assessed by considering the R^2 value and the area under the receiver operator characteristic (AUROC), and model fit compared using the LR test.

The resultant model can be used in two different ways. Firstly, the equation can be used to give an estimated probability of the predicted outcome occurring. Secondly, a dichotomous cut off can be applied, above which the outcome is taken to be a positive result (for example FDIU). Once the combination of variables with the best predictive ability was identified, sensitivity/specificity was plotted against probability cut off to investigate where the dichotomous cut off value should be to maximise model performance. Different cut off values were tested, and the corresponding sensitivity/specificity and positive/negative predictive values calculated to determine the most appropriate cut off point. statistics were calculated to look at the agreement between predicted and observed risks. The observed/expected ratio was reported for groups of predicted risks increasing in 0.1 increments, and the calibration-in-the-large (CITL) and calibration slope statistics calculated using the Stata package pmcalplot (197). To determine how well participants who do and do not develop the outcome of interest are discriminated between, the Concordance (C)-statistic was calculated. Given that the models proposed here rely on logistic regression, the C-statistic is equal to the AUROC.

Internal validation of the model was performed to evaluate the potential for overfitting and determine if any adjustments for optimism need to be made. To maximise the number of cases in the test cohort, the decision was made not to split the cohort into a test and training cohort, therefore internal validation was undertaken using bootstrapping to retain the original sample size for model development. Bootstrapping is performed by sampling *n* individuals with replacement from the original data to obtain a new sample the same size as the original data (the bootstrap sample). The same modelling and selection methods that were used to build the original model are applied to the bootstrap sample. The performance of the bootstrap model on the bootstrap sample is calculated (e.g. C-statistic, calibration slope), and the performance of the bootstrap model is also

tested in the original sample. Optimism is calculated for each statistic as a measure of the difference between the performance in the bootstrap sample and the performance in the original sample. The process was repeated 500 times to take 500 bootstrap samples and the average of the 500 optimism estimates calculated. For each performance statistic, the average optimism was subtracted from the apparent performance calculated prior to the bootstrapping, to give an optimism-corrected performance for the original model. To produce the final model, the coefficients need to be adjusted for optimism. The optimism-adjusted calibration slope can be taken as the uniform shrinkage factor, and the original model coefficients were multiplied by this value.

3.2.6. Prediction of gestation at delivery / birthweight

Linear regression with a generalised linear model assuming a Gaussian family and identity link was performed with gestational age at delivery and birthweight as the dependent variables, and maternal characteristics/ultrasound parameters as the independent variables. Estimates were also adjusted for gestational age at time of the diagnosis. Results are reported as coefficients with 95% confidence intervals. P values are reported, with a P value of < 0.05 taken to be statistically significant.

3.2.7. Longitudinal data analysis

3.2.7.1. Longitudinal weight change

For this section of the analysis, St Mary's data were combined with data obtained from East Lancashire Teaching Hospitals NHS Trust. ELHT is a large secondary care maternity unit serving an ethnically diverse population in the North West of England with an above average level of economic deprivation. Gestational age at scan (days), EFW (Hadlock BPD-HC-AC-FL), gestational age at delivery (days), birthweight and pregnancy outcome were extracted from the main database. Doppler scans, and therefore eFGR cases where only a Doppler scan was performed, were excluded from this segment of the analysis. Growth scans were labelled sequentially by gestational age at scan. Gestational age at delivery and birthweight were included as the final endpoints of the pregnancy.

Multilevel mixed effects linear regression analysis (including both fixed and random effects) was used to analyse the growth trajectory throughout pregnancy. The fixed effect component included gestational age, pregnancy outcome (as per the two groups described in Section 3.2.5.) and the first order interaction between gestational age and pregnancy outcome. The random effect component included the intercept and the linear effects of gestational age. Repeat measurements at different weeks of gestation within the same case constituted level 1 and level 2 was represented by individual cases. Regression analysis was performed using both linear and up to third order polynomial regression to determine the best fit. Analysis was repeated both with and without pregnancy outcome included as an interaction term to investigate if this affected the relationship between gestational age and EFW (i.e. the growth trajectory).

3.2.7.2. Weight gain as a prognostic factor

Following confirmation that growth trajectory is related to pregnancy outcome, the use of fetal weight gain as a prognostic factor was subsequently explored. To investigate the weight gain at different stages of pregnancy, gestation was split into two-week epochs: < 24^{+0}/40, 24^{+0} – 25^{+6}/40,

26^{+0} – 27^{+6}/40, 28^{+0} – 29^{+6}/40, 30^{+0} – 31^{+6}/40, $\geq 32^{+0}$/40. For each case in the cohort, the weekly weight gain over each gestational time period was calculated. This variable was then used as the independent variable in a logistic regression, to determine if the weight gain in each time period was related to the pregnancy outcome. Logistic regression was then repeated, adjusting for the EFW at the time of the scan. If the coefficient for the weight gain variable was found to be significant then it was concluded that growth rate during that particular gestational age bin was predictive of outcome. Following this, a new variable was created to show weight gain by a factor of 50, and logistic regression used to determine how a 50g weekly weight gain affected the risk of adverse pregnancy outcome. Analysis was performed for each pregnancy outcome combination (live birth/FDIU and survival to discharge/neonatal death).

3.2.7.3. Longitudinal Doppler change

The method of analysis applied to assess longitudinal weight gain was also used to investigate longitudinal UtA Doppler change and longitudinal UA Doppler change. The same methods were used, but UtA/UA PI was substituted for EFW. Doppler-only scans as well as growth scans were included for this section of the analysis.

3.2.8. Accuracy of estimated fetal weight estimation

Birthweight is an important predictor of survival (73). As EFW in the antenatal period is predictive of birthweight, EFW is also an important prognostic factor. EFW is influenced by both scan error and the performance of the models used to calculate it. A subset of the cohort was used to determine the error in EFW calculation in eFGR using multiple previously published sonographic weight estimation models, including some specifically developed for FGR/SGA pregnancies, and to determine if accuracy was influenced by external factors. Cases included in this analysis presented to the translational research clinics from June 2009 to September 2018, and only those pregnancies where the fetus was alive at the time of scan and had a growth scan performed within 48 hours of delivery were included (n = 65). Twenty-one previously published sonographic fetal weight estimation models were identified from the literature (Table 3.2) and investigated to determine which is the most appropriate model to use in this cohort of patients. This included the Hadlock BPD-HC-AC-FL model (198), which is currently used as standard practice in the Research Clinics at St Mary's Hospital.

Table 3.2: Sonographic fetal weight estimation models selected for investigation.

Model number	Equation	Reference
BPD, HC, AC, FL		
1	Log EFW (g) = 1.3596 + 0.0064*HC + 0.0424*AC + 0.174*FL + 0.00061*BPD*AC - 0.00386*AC*FL	Hadlock, 1985 (198)
HC, AC, FL		
2	Log EFW (g) = 1.326 - 0.00326*AC*FL + 0.0107*HC + 0.0438*AC + 0.158*FL	Hadlock, 1985 (198)
3	Ln EFW (g) = 0.04355*HC + 0.05394*AC - 0.0008582*HC*AC + 1.2594*(FL/AC) - 2.0661	Ott, 1986 (199)
4	Log EFW (g) = $0.23718*AC^2*FL + 0.03312*HC^3$	Combs, 1993 (200)
BPD, AC, FL		
5	Log EFW (g) = 1.335 - 0.0034*AC*FL + 0.0316*BPD + 0.0457*AC + 0.1623*FL	Hadlock, 1985 (198)
BPD, AC		
6	Log EFW (g) = $1.1134 + 0.05845*AC - 0.000604*AC^2 - 0.007365*BPD^2 + 0.000595*BPD*AC + 0.1694*BPD$	Hadlock, 1984 (201)
7	Log EFW (g) = $-1.599 + 0.144*BPD + 0.032*AC - 0.000111*BPD^2*AC$	Warsof, 1977 (202)
8	Log EFW (g) = -1.7492 + 0.166*BPD + 0.046*AC - 0.002546*AC*BPD	Shepard, 1982 (203)
AC, FL		
9	Log EFW (g) = 1.304 + 0.05281*AC + 0.1938*FL - 0.004*AC*FL	Hadlock, 1985 (198)
10	Ln EFW (g) = $2.792 + 0.108*FL + 0.0036*AC^2 - 0.0027*FL*AC$	Warsof, 1986 (204)
11	Ln EFW (g) = $0.77125 + 0.13244*AC - 0.12996*FL - 1.73588*AC^2/1000 + 3.09212*FL*AC/1000 + 2.18984(FL/AC)$	Ferrero, 1994 (95)
HC, AC		
12	Log EFW (g) = $5.084820 – 54.06633*AC^3 – 95.80076*AC^3*\log_{10}(AC) + 3.136370*HC$	Stirnemann, 2017 (96)
AC		
13	Ln EFW (g) = $-4.564 + 0.282*AC - 0.00331*AC^2$	Campbell, 1975 (93)
14	EFW (g) = $0.0816*AC^3$	Higginbottom, 1975 (94)
15	Log EFW (g) = $-1.8367 + 0.092*AC - 0.000019*AC^3$	Warsof, 1977 (202)
16	Ln EFW (g) = $2.695 + 0.253*AC - 0.00275*AC^2$	Hadlock, 1984 (201)
FL		
17	Ln EFW (g) = $4.6914 + 0.151*FL^2 - 0.0019*FL^3$	Warsof, 1977 (202)
SGA specific models		
HC, AC, FL		
18	Log EFW (g) = $0.66*\log_{10}HC + 1.04*\log_{10}AC + 0.985*\log_{10}FL$	Scott, 1996 (<1000g) (205)
19	EFW (g) = $5381.193 + 150.324*HC + 2.069*FL^3 + 0.0232*AC^3 - 6235.478[\log_1 HC]$	Schild, 2004 <1600g (206)
BPD, AC, FL		
20	EFW (g) = $-5498.336 + 2101.261*\ln(AC) + 15.613*FL^2 + 0.0577*BPD^3$	Siemer, 2009 (<2500g) (207)
BPD, AC		
21	EFW (g) = 9.337*BPD*AC - 229	Thurnau, 1983 (<2500g) (208)

3.2.8.1. Statistical analysis

The published coefficients for each model were used to calculate the EFW for each case in the cohort according to each model. The calculated EFW was then used to calculate the error, expressed as a percentage of the actual birthweight:

$$Percentage\ error = \frac{EFW - birthweight}{birthweight} \times 100$$

For each model, the systematic error (mean percentage error) and random error (standard deviation of percentage error * 100) were calculated as measures of accuracy and precision respectively. These were plotted for each model as a box and whisker plot. This was repeated with the cohort split according to gestational age (<28/≥28 weeks' gestation), EFW (<750g/≥750g), fetal presentation (cephalic/breech) and fetal asymmetry (HC:AC </≥95th centile (93)) to determine if accuracy and precision were influenced by these parameters.

A perfect model would have a systematic error of 0% and a random error of 0%, therefore by creating a scatter plot of systematic error against random error, and ranking models according to their distance from the origin (0, 0), the best performing model can be identified. Distance from the origin was calculated as a composite of the random and systematic errors using Pythagoras' theorem:

$$Distance\ from\ origin = \sqrt{Random\ error^2 + Systematic\ error^2}\ .$$

With the best performing model, regression analysis was used to investigate the relationship between mean percentage error for each case and gestational age at the time of scan, EFW and amniotic fluid index (AFI) to determine if these continuous variables influenced the model performance.

3.3. Results

In total, 278 pregnancies were identified from St Mary's Hospital during the study period, 182 of which met the eligibility criteria as eFGR, as summarised in Figure 3.2.

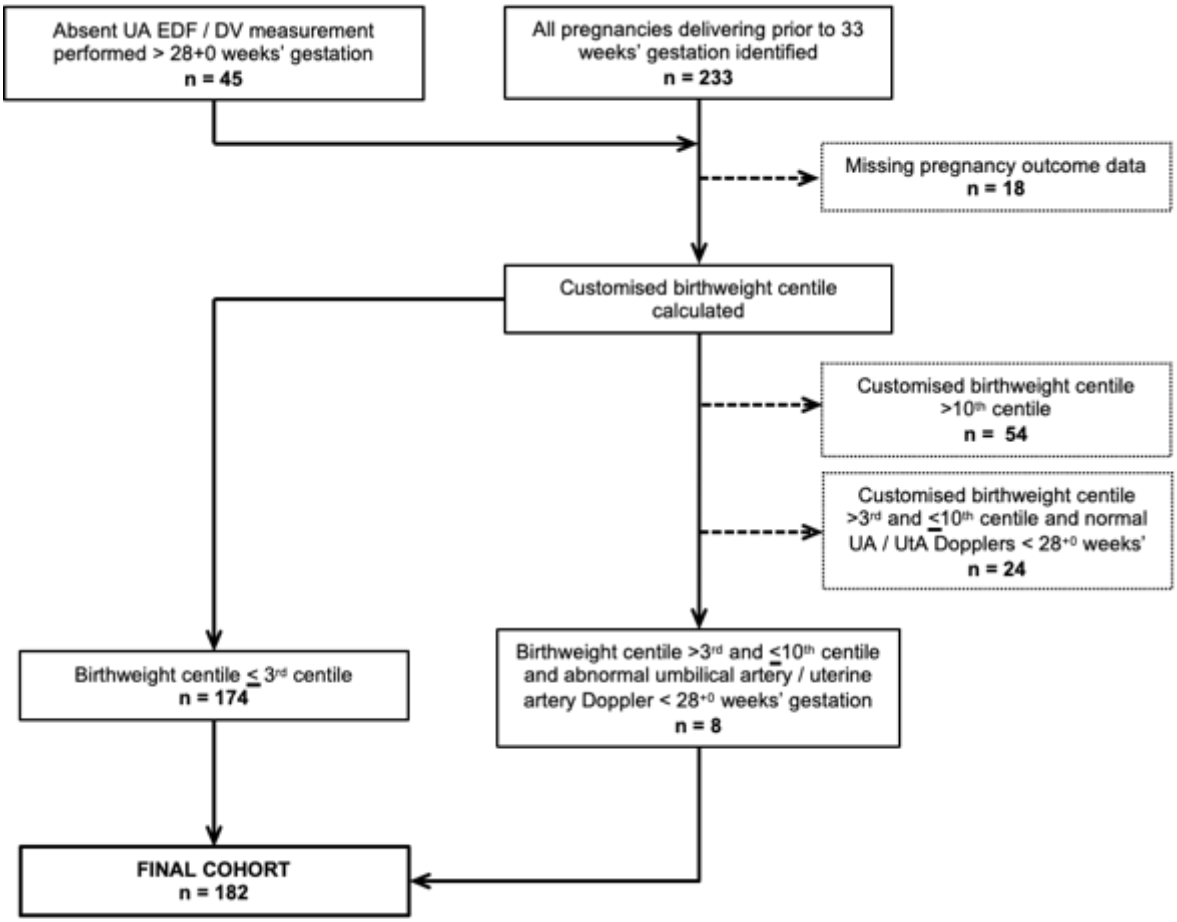


Figure 3.2: Flow chart to summarise how final cohort was derived from initial data collected

To characterise the cohort (Section 3.3.1), data are summarised firstly as the whole cohort, and is then split by pregnancy outcome (survived to discharge/FDIU/neonatal or infant death). For the predictive factors, analysis was performed by two outcome measures: firstly, live birth compared to FDIU, and secondly survived to discharge compared to overall death (composite of FDIU and neonatal/infant death). For extremely skewed values, data were also logarithmically transformed, and normality tested using the resulting values.

3.3.1. Characterisation of cohort

3.3.1.1. Maternal characteristics

The mean maternal age at presentation was 31.1 years (SD 6.0 years), and the median BMI was 27.2 (range 16.1-46.6). In those with booking blood pressure (BP) data available (n = 82), 12% of the whole cohort were classed as hypertensive at booking (BP > 140/90). Just under half of the cohort were White British (n = 88; 49%), and just over half were primiparous (n = 100; 55%). Maternal demographic data are summarised in Table 3.3. When split according to pregnancy outcome, only mean arterial pressure (MAP) (driven by a difference in the diastolic BP) showed a statistically significant difference between pregnancy outcomes, with those pregnancies ending in FDIU having a higher MAP at booking.

Table 3.3: Maternal characteristics at booking. Data are displayed for the whole eFGR cohort, then split according to pregnancy outcome. Significance column refers to comparisons between the three pregnancy outcomes.

Variable	Total cohort (n = 182)	Live birth, survived to discharge (n = 111)	FDIU (n = 45)	Neonatal/infant death (n = 26)	Significance
Age (years)*	31.1 ± 6.0	31.1 ± 5.7	32.2 ± 6.0	29.1 ± 6.9	ns (P = 0.12)
Booking weight (kg)†	70.2 (33-127)	73 (43-127)	70 (33-127)	67 (48-104)	ns (P = 0.69)
Booking height (cm)*	162.4 ± 6.7	162.5 ± 6.2	161.8 ± 8.5	163.4 ± 5.2	ns (P = 0.70)
Booking BMI (kg/m^2)†	27.2 (16.1-46.6)	27.3 (17.0-43.9)	26.7 (16.2-46.6)	25.8 (18.3-40.6)	ns (P = 0.55)
Missing	34	18	10	6	
Maternal booking blood pressure (mmHg)†					
Systolic	120 (90-170)	110 (90-160)	120 (100-170)	126 (90-138)	ns (P = 0.20)
Diastolic	70 (50-109)	70 (50-102)	80 (58-109)	70 (58-90)	P = 0.05
MAP	84.7 (63.3-127.3)	84.0 (63.3-120.0)	98.8 (72.0-127.3)	82.7 (71.3-106)	P < 0.001
Hypertensive at booking (>140/90)	10 (12%)	7 (13%)	3 (25%)	0 (0%)	
Normotensive at booking (≤140/90)	72 (88%)	54 (87%)	9 (75%)	9 (100%)	ns (P = 0.22)
Missing	100	50	33	17	
Smoking status‡					
Non-smoker	105 (84%)	71 (85%)	20 (77%)	14 (93%)	
Smoker	20 (16%)	13 (15%)	6 (23%)	1 (7%)	ns (P = 0.31)
Missing	57	27	19	11	
Ethnicity‡					
White	88 (49%)	52 (47%)	20 (44%)	16 (62%)	
Mixed	1 (0.6%)	0 (0%)	1 (2%)	0 (0%)	
African	20 (11%)	14 (13%)	4 ((%)	2 (8%)	
Asian	31 (17%)	19 (17%)	8 (18%)	4 (15%)	ns (P = 0.88)
Black/Caribbean	3 (2%)	2 (2%)	1 (2%)	0 (0%)	
Arabic	2 (1%)	1 (1%)	1 (2%)	0 (0%)	
Other/unknown	37 (20%)	23 (21%)	10 (22%)	4 (15%)	
Parity‡					
Primiparous	100 (55%)	54 (48.7%)	29 (64.4%)	17 (65.4%)	ns (P = 0.1)
Multiparous	82 (45%)	57 (51.4%)	16 (35.6%)	9 (34.6%)	

Previous FDIU Yes No	 18 (22%) 64 (78%)	 16 (28%) 41 (72%)	 2 (13%) 14 (87%)	 0 (0%) 9 (100%)	ns (P = 0.21)
Previous NND Yes No	 17 (21%) 65 (79%)	 13 (23%) 44 (77%)	 3 (19%) 13 (81%)	 1 (11%) 8 (89%)	ns (P = 0.91)

* Mean ± standard deviation, ANOVA; † Median (range), Kruskal-Wallis; ‡ Count (percentage), Chi-squared

3.3.1.2. Pregnancy outcome

Pregnancy outcome data are summarised in Table 3.4. The median gestational age at delivery was 29.4 weeks (range 22.0-33.0 weeks), with a median birthweight of 695g. 61% (n = 111) of cases survived to discharge from the neonatal unit, 25% unfortunately ended in FDIU and 14% resulted in a neonatal or infant death. Between pregnancy outcomes, there was a significant difference in gestational ages at delivery and birthweights/birthweight centiles, with those cases that survived to discharge unsurprisingly being born later (30.3 weeks' gestation (range 25.1-33.0)) and at higher birthweights 875g (range 450-1718g), and those cases ending in FDIU being born the earliest (26.9 weeks' (range 22-32.4) and at the smallest birthweights (390g (range 210-710g)). These data are displayed as a scatter graph (Figure 3.3).

Table 3.4: Pregnancy outcomes for the eFGR cohort. Data are displayed for the whole cohort, then split according to pregnancy outcome. Significance column refers to comparisons between the three pregnancy outcomes.

Variable	Total cohort (n = 182)	Live birth, survived to discharge (n = 111)	FDIU (n = 45)	Neonatal/ infant death (n = 26)	Significance
Gestational age at delivery (weeks)†	29.4 (22.0-33.0)	30.3 (25.1-33.0)	26.9 (22-32.4)	28.7 (25.6-31.7)	P < 0.001
Birthweight (g)†	695 (210-1718)	875 (450-1718)	390 (210-710)	595 (360-1079)	P < 0.001
Birthweight centile†	0.003 ($5e^{-6}$-9.9)	0.02 ($5x10^{-6}$-9.9)	$1x10^{-5}$ ($5x10^{-6}$-0.2)	$5x10^{-4}$ ($5x10^{-6}$-4.1)	P < 0.001
Infant sex‡ Male Female	 103 (57%) 79 (43%)	 59 (53%) 52 (47%)	 27 (60%) 18 (40%)	 17 (65%) 9 (35%)	P = 0.46
Mode of delivery‡ Vaginal Caesarean section Unknown	 46 (25%) 133 (73%) 3 (2%)	 3 (3%) 107 (96%) 1 (1%)	 45 (100%) 0 (0%) 0 (0%)	 0 (0%) 26 (100%) 0 (0%)	P < 0.001

† Median (range), Kruskal-Wallis; ‡ Count (percentage), Chi-squared

The median birthweight centiles in all outcome groups were <0.1st centile, so there is little discrimination between them on this basis, but there was still a significant difference across the groups. As expected, there was a difference in the mode of delivery, with all FDIU cases being delivered vaginally, and 96% and 100% of the survived to discharge and neonatal death cases respectively delivered by Caesarean section.

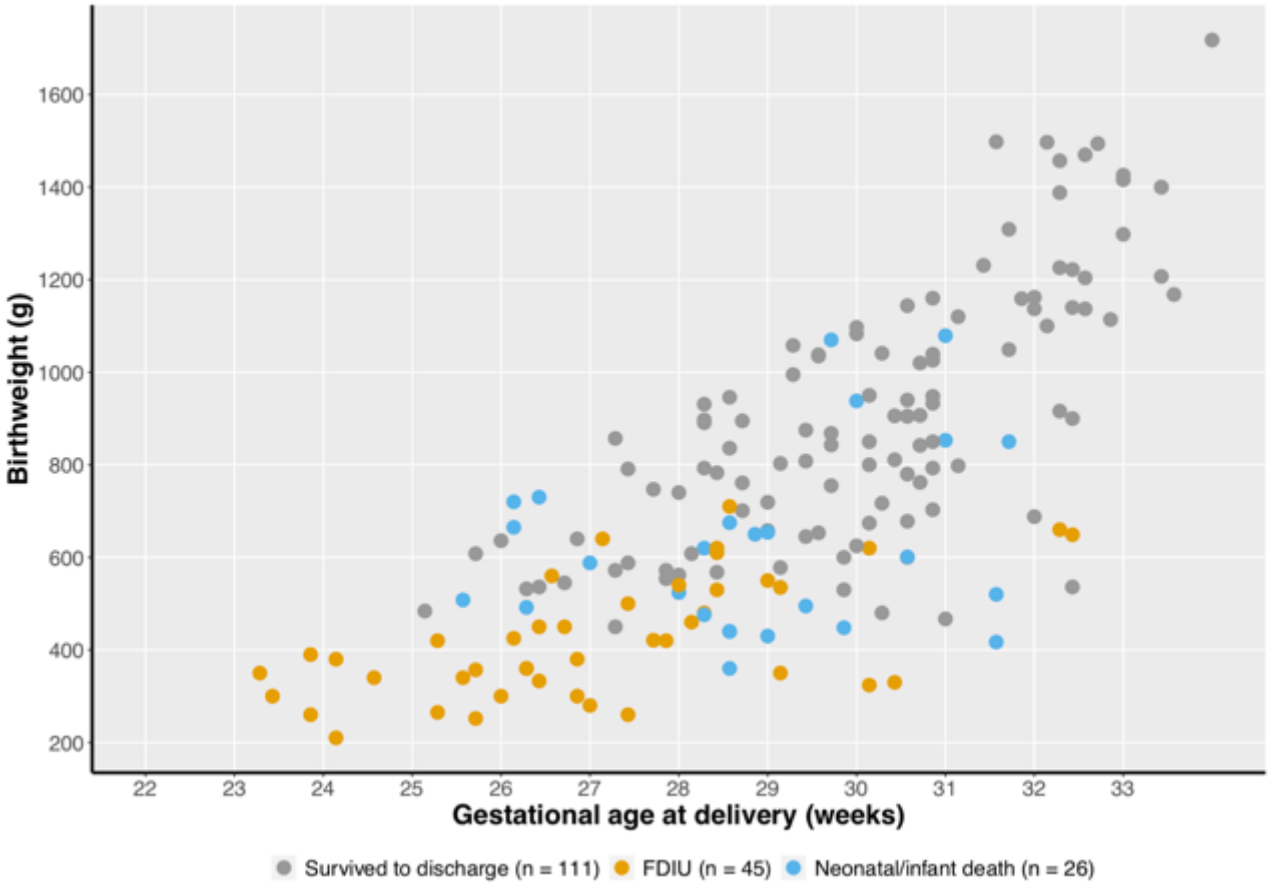


Figure 3.3: Birthweight plotted against gestation for each eFGR case, and colour coded according to pregnancy outcome. Cases which survived to discharge are skewed towards the later gestations and higher birthweights, FDIU cases are skewed towards earlier gestations and lower birthweights, and neonatal/infant death cases are throughout the mid-portion of the graph.

3.3.1.3. Neonatal outcome

Neonatal outcome data were available for all cases that were born alive (Table 3.5). The median length of stay for those infants who survived to discharge was 42 days, ranging from 2 to 369, with the case of 2 days being discharged to another unit. In those that did not survive the median time to death was 9 days, with a range from 0 to 169 days. Only one third of cases were discharged from the St Mary's Neonatal Unit to home, the remainder were discharged to another unit, either for specialist care that could not be provided at St Mary's Hospital, or to a local unit once Level 3 care was no longer required.

In terms of morbidity, rates of intraventricular haemorrhage were higher amongst those infants that died, occurring in 42% (n = 11), compared to 24% (n = 27) in those that survived (P = 0.05). There was no statistically significant difference in rate of necrotising enterocolitis (NEC) diagnosis between those that died and those that survived. In terms of management of NEC however, those that died were much more likely to have had a laparotomy rather than conservative management (3 (43%) compared to 1 (5%); P < 0.001). Interestingly, sepsis appeared much lower in those that died, with 46% (n = 12) not even having presumed sepsis, compared with 21% (n = 23) in those that survived, although this may reflect the length of neonatal stay in the two groups, with those cases ending in death much shorter on average than in those that survived. Overall, it would

appear that a diagnosis of NEC requiring active management is a poor prognostic indicator for survival, but this analysis does not have sufficiently detailed data to account for severity within these morbidity categories.

Table 3.5: Neonatal outcomes for those eFGR cases that resulted in a live birth. Data are displayed as the whole live birth cohort, then split according to outcome.

Variable	Total cohort (n = 137)	Live birth, survived to discharge (n = 111)	Neonatal/infant death (n = 26)	Significance
Time until discharge/death (days) †		42 (1-369)	9 (0-169)	
Missing		8	9	
Discharge destination‡				
Home		41 (36.9%)		
Other hospital		66 (59.5%)		
Not recorded		4 (3.6%)		
Necrotising enterocolitis‡				
Yes	29 (21%)	22 (20%)	7 (27%)	P = 0.63
No	106 (77%)	89 (80%)	19 (73%)	
Conservative management	25 (86%)	21 (95%)	4 (57%)	P = 0.03
Laparotomy	4 (14%)	1 (5%)	3 (43%)	
Chronic lung disease‡				
Yes	90 (66%)	77 (70%)	13 (50%)	P = 0.28
No	47 (34%)	34 (30%)	13 (50%)	
Retinopathy of prematurity‡				
Yes	19 (14%)	19 (17%)	0 (0%)	P = 1.00
No	118 (86%)	92 (83%)	0 (0%)	
Intraventricular haemorrhage‡				
Yes	38 (28%)	27 (24%)	11 (42%)	P = 0.05
No	99 (72%)	84 (76%)	15 (58%)	
Sepsis‡				
Presumed	80 (58%)	66 (59%)	14 (54%)	P = 0.15
Confirmed	13 (10%)	13 (12%)	0 (0%)	
No	44 (32%)	23 (21%)	12 (46%)	

† Median (range), Mann-Whitney test; ‡ Count (percentage), Chi-squared

3.3.2. Ultrasound characteristics at diagnosis

The average gestational age at diagnosis was 23.7 weeks (range 20.0-30.6) and was earlier in cases that ended in FDIU compared to cases that survived to delivery (23.0 weeks; range 20.0-29.3; compared to 24.1 weeks; range 20.3-30.6); P < 0.001). The scan that was taken to be the "diagnosis scan" was the first recorded scan after 20 weeks' gestation that included fetal biometry, maternal Dopplers and placental biometry. Unfortunately, not all of the diagnostic scans performed met all of these criteria, with some cases missing placental biometry or maternal Doppler measurements. Table 3.6 displays the total number of observations for each variable. Analysis was also repeated including only those scans that were performed between 21-24 weeks' gestation, which is the standard timeframe for performing a "placental screen" in our unit (n = 103, 71% of cases in total cohort). It would be expected that some cases would be identified later than this, due to concerns not being raised about fetal growth until after 24 weeks', or initial management taking place at a local unit, prior to referral to a specialist clinic. It was decided that the final model should be based on measurements at the time of diagnosis, rather than in a specific gestational age window, to maximise its clinical usefulness. Data are summarised in Table 3.7.

Table 3.6: Total number of observations for each variable.

Variable	Total cohort (n = 182)	Live birth, survived to discharge (n = 111)	FDIU (n = 45)	Neonatal/infant death (n = 26)
Fetal biometry				
BPD (mm)	136	77	37	22
HC (mm)	168	103	40	25
AC (mm)	173	107	40	26
FL (mm)	170	106	39	25
EFW (g)	168	103	39	25
EFW customised centile	168	103	39	25
EFW non-customised centile	168	103	39	25
Liquor volume				
Deepest pool (cm)	78	45	20	13
AFI (cm)	118	69	28	21
Fetal Dopplers				
UA PI	78	56	12	10
UA RI	69	51	10	8
UA EDF	154	92	38	24
MCA PI	71	37	23	11
MCA RI	62	34	20	8
DV PIV	45	25	13	8
DV PVIV	47	26	12	8
DV a-wave	74	37	23	14
Maternal Dopplers				
R UtA PI	131	80	32	19
R UtA RI	125	78	30	17
L UtA PI	126	77	30	19
L UtA RI	119	74	28	17
Mean UtA PI	126	77	30	17
Mean UtA RI	119	74	28	17
Notching	140	86	26	18
Placental biometry				
Largest diameter (cm)	88	54	22	12
Smallest diameter (cm)	84	51	22	11
Depth (cm)	86	53	21	12
PEC	84	51	21	11
PSA	84	51	21	11
Gestational age (weeks)	182	111	45	26
Blood pressure				
Systolic (mmHg)	54	38	11	5
Diastolic (mmHg)	54	38	11	5

Table 3.7: Summary of ultrasound characteristics at the time of diagnosis of eFGR. Data are displayed as the whole cohort, and then split according to pregnancy outcome.

Variable	Total cohort (n = 182)	Live birth, survived to discharge (n = 111)	FDIU (n = 45)	Neonatal/infant death (n = 26)	Significance
Fetal biometry					
BPD (mm) †	53.7 (26.3-81.9)	55.3 (38.6-81.9)	48.7 (26.3-66.9)	55.9 (38.9-75.4)	P < 0.001
HC (mm) †	201.1 (143.2-289.4)	205.9 (143.2-289.4)	183.3 (152.0-238.9)	202.1 (151.8-253.9)	P < 0.001
AC (mm)*	170.3 ± 28.9	179.5 ± 27.7	148.7 ± 18.6	165.4 ± 28.5	P < 0.001
FL (mm)*	36.3 ± 7.5	38.7 ± 7.1	31.1 ± 5.5	34.6 ± 7.9	P < 0.001
EFW (g) †	460 (153-1371)	520 (153-1371)	321 (166-682)	472 (157-862)	P < 0.001
EFW customised centile†	0.4 (0.0-73.5)	1.1 (0.0-73.5)	0.02 (0.0-36.2)	0.2 (0.0-45.2)	P < 0.001
EFW non-customised centile†	0.4 (0.0-56.5)	1.7 (0.0-56.5)	0.01 (0.0-12.2)	0.1 (0.0-34.7)	P < 0.001
Liquor volume					
Deepest pool (cm)*	3.5 ± 1.4	3.8 ± 1.3	2.8 ± 1.4	3.7 ± 1.4	P = 0.02
AFI (cm)*	9.45 ± 3.9	10.2 ± 3.8	7.6 ± 3.0	9.5 ± 4.4	P = 0.01
Fetal Dopplers					
UA PI†	1.5 (0.8-3.3)	1.5 (0.8-3.3)	1.8 (1.1-3.3)	1.6 (1.0-2.4)	P = 0.02
UA RI†	0.8 (0.6-1.3)	0.8 (0.6-1.1)	0.9 (0.7-1.3)	0.8 (0.7-1.0)	ns (P = 0.06)
UA EDF‡					
Present	60 (39.0%)	50 (54.4%)	4 (10.5%)	6 (25.0%)	
Absent	86 (55.8%)	40 (43.5%)	29 (76.3%)	17 (70.8%)	P < 0.001
Reversed	8 (5.2%)	2 (2.2%)	5 (13.2%)	1 (4.2%)	
MCA PI†	1.4 (0.9-2.0)	1.4 (0.9-1.8)	1.3 (0.9-1.8)	1.1 (1.0-2.0)	ns (P = 0.06)
MCA RI*	0.7 ± 0.1	1.4 ± 0.2	0.7 ± 0.1	0.69 ± 0.1	P = 0.05
DV PIV†	0.9 (0.4-2.7)	0.94 (0.5-1.3)	0.9 (0.5-1.2)	0.7 (0.4-2.7)	ns (P = 0.43)
DV PVIV†	0.8 (0.4-1.8)	0.8 (0.4-1.8)	0.7 (0.5-1.4)	0.7 (0.4-1.5)	ns (P = 0.74)
DV a-wave ‡					
Present	65 (88%)	36 (97%)	17 (74%)	12 (86%)	
Absent	7 (9%)	1 (3%)	5 (22%)	1 (7%)	P = 0.02
Reversed	2 (3%)	0 (0%)	1 (4%)	1 (7%)	
Maternal Dopplers					
R UtA PI†	1.5 (0.5-3.6)	1.5 (0.5-3.6)	1.6 (0.8-3.5)	1.2 (0.5-2.8)	ns (P = 0.11)
R UtA RI†	0.7 (0.2-0.9)	0.7 (0.4-0.9)	0.7 (0.5-0.9)	0.6 (0.2-0.9)	ns (P = 0.07)
L UtA PI†	1.6 (0.5-4.2)	1.5 (0.6-3.5)	1.7 (0.8-4.2)	1.5 (0.5-2.5)	ns (P = 0.31)
L UtA RI†	0.7 (0.4-0.9)	0.7 (0.4-0.9)	0.8 (0.6-0.9)	0.7 (0.4-0.9)	ns (P = 0.07)

Mean UtA PI*	1.6 ± 0.5	1.6 ± 0.5	1.7 ± 0.6	1.4 ± 0.5	ns (P = 0.15)
Mean UtA RI†	0.7 (0.4-0.9)	0.7 (0.4-0.8)	0.7 (0.6-0.9)	0.7 (0.4-0.8)	P = 0.03
Notching‡					
No notch	44 (31%)	30 (35%)	10 (28%)	4 (22%)	
Unilateral notch	28 (20%)	13 (15%)	10 (28%)	5 (28%)	ns (P = 0.56)
Bilateral notch	68 (49%)	43 (50%)	16 (44%)	9 (50%)	
Placental biometry					
Largest diameter (cm)*	10.6 ± 1.9	10.9 ± 1.7	9.9 ± 1.8	10.3 ± 2.2	ns (P = 0.1)
Smallest diameter (cm)*	9.7 ± 2.1	10.2 ± 1.9	8.6 ± 2.3	9.9 ± 1.9	P = 0.008
Depth (cm) †	3.6 (1.6-8.0)	3.8 (1.6-7.8)	3.6 (2.5-7.6)	2.8 (2.2-8.0)	ns (P = 0.47)
PEC†	28.7 (7.2-89.0)	33.2 (8.9-89.0)	20.0 (9.1-42.8)	34.4 (7.2-51.5)	P = 0.02
PSA*	104.9 ± 35.4	113.3 ± 34.2	86.6 ± 34.1	102.6 ± 31.2	P = 0.01
Gestational age (weeks)	23.7 (20-30.6)	24.1 (20-30.6)	23.0 (20-29.3)	24.1 (20.3-29.4)	P < 0.001
Blood pressure					
Systolic (mmHg)†	144 (90-174)	143 (90-164)	149 (122-171)	165 (126-174)	ns (P = 0.12)
Diastolic (mmHg)†	90.5 (60-113)	89 (60-108)	90 (74-106)	102 (67-113)	ns (P = 0.38)

* Mean ± standard deviation, ANOVA; † Median (range), Kruskal-Wallis; ‡ Count (percentage), Chi-squared

There was a significant difference across the outcome groups between EFW, EFW centile and each fetal biometry measurement (BPD, HC, AC, FL), with the cases ending in FDIU being the smallest at diagnosis and those that survived being the largest. AFI was measured in 118 cases and was also different between outcomes, with those pregnancies ending in FDIU having significantly less liquor at diagnosis (P = 0.01). Fewer cases had a recorded SDVP available (n = 78), but this showed the same trend.

In terms of fetal Dopplers, over half of the cohort had absent EDF at the time of diagnosis. This differed between the outcome groups, with significantly more cases ending in death presenting with absent EDF (73% compared to 38%; P <0.001). In those where a PI measurement could be taken (n = 78), the median UA PI across all cases was 1.5 (range 0.8-3.3); a UA PI of 1.5 represents the 95% centile at 23 weeks' gestation (209), which highlights the extreme nature of this cohort, even at the point of diagnosis. In cases ending in FDIU, the median UA PI at diagnosis was 1.78 (range 1.14-3.33), with 77% (n = 29) of cases having absent UA EDF. This is in contrast to those that were born alive, who had a median UA PI of 1.46 (range 0.83-3.31), with 38% having absent EDF at presentation. Values for the MCA did not show a significant difference across the groups. There did appear to be a trend towards lower values in cases ending in neonatal/infant death, however the number in this group was small (n = 11) and so any conclusions potentially inaccurate. The numbers of patients across the whole cohort with a value recorded for DV PIV/peak velocity index for veins (PVIV) was also small (n = 45 and n = 47 respectively), and no difference was seen across the groups for these parameters. However, there was a difference in the a-wave recorded at diagnosis, with one-fifth (n = 5) of cases ending in FDIU having an absent a-wave, compared with 2.7% (n = 1) in those born alive (P = 0.01), although the total number of cases with an abnormal a-wave at diagnosis is too small to draw any robust conclusions from (n = 9; 12% of those recorded).

With reference to maternal Dopplers, there is a non-significant trend towards a lower mean UtA PI in cases ending in neonatal/infant death, with the mean across the whole cohort being 1.6 (SD 0.5). The 95th centile for UtA PI at 23 weeks is 1.41 (108), suggesting that across the cohort, UtA Dopplers were abnormal. When considering the presence of unilateral/bilateral UtA notching there was no difference between the different outcome groups, with 50% of the cohort (n = 68) having bilateral notching at diagnosis. As previously mentioned, the most likely placental histopathological finding in this cohort of eFGR is MVM, with which abnormal UtA Dopplers would be associated.

Placental biometry was found to be different between the outcome groups, with PEC (P = 0.02) and PSA (P = 0.01) both smaller in those cases ending in FDIU, likely driven by a smaller minimum diameter (P = 0.008).

Finally, BP data at the time of the scan was available in 54 cases in the cohort. Although too small a number to draw any robust conclusions from, it does suggest a trend towards a higher BP across all cases, but particularly in those that ended in neonatal/infant death, where the median BP was

165 (range 126-174) /102 (range 67-113). Overall, BP at the time of diagnosis was 140 (SD 18.4)/91 (60 – 113), which suggests an increasing trend from the recorded BP at booking.
If each pregnancy outcome is considered separately, there are some characteristics that appear to be shared between different outcome groups. For example, in terms of fetal biometry, liquor volume and placental biometry, the group that survived to discharge and the neonatal/infant death group were more comparable to one another than the FDIU group. With fetal Dopplers, there is a continuum, with the worst Dopplers seen in the FDIU group and the best profile in the survived to discharge group. Maternal UtA Dopplers were lowest in those pregnancies ending in neonatal/infant death. BP at the time of scan, although not significant, shows a trend towards higher readings in these pregnancies.

Using only those cases where the diagnosis scan occurred between 21 and 24 weeks' gestation (the time of a "placental screen" in this tertiary unit), the differences across the three outcome groups follow the same pattern as described in Table 3.8.

Table 3.8: Summary of ultrasound characteristics at the time of the 21-24 week placental screen. Data are displayed as the whole cohort, and then split according to pregnancy outcome.

Variable	Total cohort (n = 103)	Live birth, survived to discharge (n = 57)	FDIU (n = 32)	Neonatal/infant death (n = 14)	Significance
Fetal biometry					
BPD (mm)†	51.5 (26.3-57.6)	53.0 (42.1-57.6)	48.1 (26.3-53.2)	55.5 (38.9-57.0)	P < 0.001
HC (mm)†	192.0 (151.8-219.0)	195.1 (160.0-219.0)	181.8 (152.0-198.6)	199.2 (151.8-214.3)	P < 0.001
AC (mm)*	157.9 ± 19.5	163.7 ± 18.0	147.8 ± 15.5	156.1 ± 15.5	P = 0.001
FL (mm)*	33.0 ± 5.6	34.7 ± 5.1	30.4 ± 4.3	30.4 ± 4.3	P = 0.002
EFW (g)†	387 (157-679)	440 (170-679)	318 (166-483)	425 (157-575)	P < 0.001
EFW customised centile†	0.7 ($2x10^{-6}$-73.5)	3.6 ($2x10^{-6}$ - 73.4)	0.03 ($7x10^{-6}$-36.2)	0.4 ($3x10^{-6}$-15.1)	P < 0.001
EFW non-customised centile†	0.7 (0.000004-56.5)	3.6 (0.00001-56.5)	0.03 (0.00001-12.2)	0.2 (0.000004-18.3)	P < 0.001
Liquor volume					
Deepest pool (cm)*	3.5 ± 1.4	3.8 ± 1.4	2.8 ± 1.1	4.4 ± 1.5	P = 0.02
AFI (cm)*	10.0 ± 3.9	10.8 ± 4.0	8.1 ± 3.1	11.2 ± 3.4	P = 0.02
Fetal Dopplers					
UA PI†	1.5 (1.0-3.3)	1.4 (1.0-2.0)	1.8 (1.1-3.3)	1.6 (1.0-2.0)	P = 0.004
UA RI†	0.8 (0.6-1.3)	0.8 (0.6-0.9)	1.0 (0.7-1.3)	0.8 (0.7-1.0)	P = 0.01
UA EDF‡					
Present	41 (46.1%)	33 (68.8%)	3 (10.7%)	5 (38.5%)	
Absent	45 (50.6%)	15 (31.3%)	22 (78.6%)	8 (61.5%)	P < 0.001
Reversed	3 (3.4%)	0 (0%)	3 (10.7%)	0 (0%)	
MCA PI†	1.3 (0.9-1.8)	1.4 (1.2-1.7)	1.4 (0.9-1.8)	1.2 ± 0.2	ns (P = 0.19)
MCA RI*	0.7 ± 0.07	0.7 ± 0.1	0.7 ± 0.07	0.6	ns (P = 0.07)
DV PIV†	1.0 (0.4-1.3)	1.0 (0.7-1.3)	0.9 (0.6-1.2)	0.6 (0.4-1.1)	ns (P = 0.08)
DV PVIV†	0.8 (0.4-1.4)	0.8 (0.7-0.8)	0.8 (0.5-1.4)	0.6 (0.4-0.9)	ns (P = 0.08)
DV a-wave‡					
Present	26 (86.7%)	8 (100%)	12 (75.0%)	6 (100%)	
Absent	4 (13.3%)	0 (0%)	4 (25.0%)	0 (0%)	ns (P = 0.2)
Reversed	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Maternal Dopplers					
R UtA PI†	1.5 (0.7-3.0)	1.5 (0.7-2.6)	1.6 (0.8-3.0)	1.3 (0.7-2.8)	ns (P = 0.8)
R UtA RI†	0.7 (0.2-0.9)	0.7 (0.4-0.9)	0.8 (0.5-0.9)	0.7 (0.2-0.9)	ns (P = 0.5)
L UtA PI†	1.6 (0.5-2.8)	1.6 (0.6-2.8)	1.7 (1.0-2.8)	1.5 (0.5-2.3)	ns (P = 0.2)

L UtA RI†	0.7 (0.4-0.9)	0.7 (0.4-0.9)	0.8 (0.6-0.9)	0.7 (0.4-0.8)	ns (P = 0.1)
Mean UtA PI*	1.6 ± 0.5	1.5 ± 0.4	1.7 ± 0.5	1.4 ± 0.5	ns (P = 0.4)
Mean UtA RI†	0.7 (0.5-0.9)	0.7 (0.5-0.8)	0.8 (0.6-0.9)	0.7 (0.5-0.8)	ns (P = 0.1)
Notching‡					
No notch	18 (23%)	11 (26%)	6 (23.1%)	1 (10%)	
Unilateral notch	17 (22%)	7 (16%)	6 (23.1%)	4 (40%)	ns (P = 0.6)
Bilateral notch	44 (56%)	25 (58%)	14 (53.9%)	5 (50%)	
Placental biometry					
Largest diameter (cm)*	10.9 ± 1.8	11.1 ± 1.9	10.3 ± 1.8	11.2 ± 1.5	ns (P = 0.3)
Smallest diameter (cm)*	9.8 ± 2.2	10.3 ± 2.0	8.5 ± 2.4	10.4 ± 1.4	P = 0.02
Depth (cm) †	3.5 (1.6-8.0)	3.5 (1.6-6.0)	3.6 (2.5-7.6)	2.6 (2.2-8.0)	ns (P = 0.4)
PEC†	32.1 (8.9-86.9)	35.7 (8.9-86.9)	20.4 (14.2-42.8)	39.2 (15.7-51.5)	P = 0.02
PSA*	109.4 ± 37.5	117.7 ± 37.6	89.9 ± 36.9	116.5 ± 20.6	P = 0.03
Gestational age (weeks)	23.1 (21-24.9)	23.1 (21-24.9)	23.0 (21-24.9)	23.6 (21.4-24.7)	ns (P = 0.3)
Blood pressure					
Systolic (mmHg)†	150 (110-174)	145 (112-164)	154 (110-171)	172 (126-174)	ns (P = 0.3)
Diastolic (mmHg)†	93 (67-113)	93 (75-108)	93 (74- 113)	102 (67-113)	ns (P = 0.5)

*Mean ± standard deviation, ANOVA; † Median (range), Kruskal-Wallis; ‡ Count (percentage), Chi-squared

3.3.2.1. *Predictive factors: Pregnancy outcome*

Table 3.9 shows both univariable and gestation-adjusted regression analysis to predict FDIU and death (composite of FDIU and neonatal/infant death) using maternal characteristics and parameters obtained from the ultrasound scan at the time of diagnosis.

On univariable analysis there was a statistically significant association between odds of both FDIU and death and increased booking systolic and diastolic BP and increased MAP. This relationship persisted when adjusted for BMI and maternal age, suggesting that these factors have independent prognostic value to predict death, with each 5 unit increase in systolic BP at booking conferring an estimated 20% increase in the risk of death (adjusted OR (aOR) 1.20 (95% CI 1.02-1.42), P = 0.03), and a 5 unit increase in booking diastolic BP increasing the risk of death by an estimated 34% (aOR 1.34 (95% CI 1.03-1.75), P = 0.03). Multiparous women had a 53% reduced chance of FDIU and overall death (OR 0.47 (0.26-0.87), P = 0.02). None of the other maternal characteristics showed any statistically significant associations with pregnancy outcome.

Each individual fetal biometric parameter and EFW was significantly associated with pregnancy outcome, which persisted when adjusting for gestational age at the time of the ultrasound scan. For example, each 25g increase in EFW was associated with a 29% reduction in the risk of FDIU (aOR 0.71; 95% CI 0.62-0.81; P < 0.001) and a 25% reduction in the risk of overall death (aOR 0.75; 95% CI 0.67-0.83; P < 0.001). A 1cm increase in AFI conferred a 21% reduction in the risk of FDIU and a 15% reduction in overall death (FDIU: aOR 0.79; 95% CI 0.68-0.92; P = 0.02; death: aOR 0.85; 95% CI 0.76-0.95; P = 0.004).

In terms of fetal Doppler indices, the parameter that shows the most promise as a prognostic factor is the UA. If this is used as a continuous variable, a 0.1 unit increase in gestation-adjusted UA PI increases the risk of both FDIU and death by 17% (FDIU aOR: 1.17; 95% CI 1.03-1.33; P = 0.01; death: aOR 1.17; 95% CI 1.03-1.32, P = 0.02). Using EDF status as a categorical variable, having absent or reversed EDF compared to present at the time of diagnosis increases the risk of FDIU by 13.4 times (aOR 13.4; 95% CI 3.7-48.6; P < 0.001), and 9 times for death (aOR 9.0; 95% CI 3.65-22.2; P < 0.001). MCA PI also showed a significant association with death, but as previously highlighted, numbers in this category were small and confidence intervals wide. DV parameters were available in less than half of the cohort (n = 74, 41%), but no significant associations were seen with either PIV/PVIV or the a-wave status and pregnancy outcome.

Interestingly, the only maternal Doppler parameter with prognostic value was the association between the mean UtA RI and FDIU, with a 0.1 increase in UtA RI associated with a two-fold increase in FDIU (aOR 2.05; 95% CI 1.23-3.43; P = 0.006). None of the other parameters were found to be significantly prognostic of outcome, most likely because UtA parameters were abnormal in the majority of cases, and so other variables lost their discriminatory ability.

Both PEC and PSA showed a significant association with both FDIU and death, independent of gestational age at measurement. This is likely driven by the smallest placental diameter, which was also associated with both outcomes. It should be noted that numbers were relatively small in this analysis (n = 84; 46%).

The relationship between maternal BP at the time of the scan and death was approaching significance when adjusted for gestational age, suggesting that every 5 unit increase in systolic BP at the time of scan increases the risk of death by 24% (aOR 1.24; 95% CI 0.98-1.57; P = 0.07), and diastolic BP by 35% (aOR 1.35; 95% CI 0.95-1.91; P = 0.09). Maternal BP was not found to be predictive of FDIU however, but these data were only available for 30% of the cohort.

There was no significant relationship between infant sex and outcome (either FDIU or death). Although sex is technically an outcome variable, if found to be prognostic of outcome it could be determined antenatally and used in a clinical prediction model.

Table 3.9: Univariable and gestation-adjusted analysis for prediction of FDIU and overall death at the time of eFGR diagnosis. Significant associations (P<0.05) are highlighted in bold.

Variable	N	Prediction of FDIU				Prediction of overall death (FDIU or neonatal/infant death)			
		Univariable analysis		Gestation-adjusted		Univariable analysis		Gestation-adjusted	
		Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
Age	182	1.04 (0.99 - 1.01)	0.09			1.01 (0.96 - 1.06)	0.80		
Booking weight (kg)	147	0.99 (0.97 - 1.02)	0.58			1.00 (0.98 - 1.01)	0.65		
Booking height (cm)	148	0.99 (0.94 - 1.05)	0.84			1.00 (0.95 - 1.05)	0.95		
BMI (kg/m^2)	147	0.98 (0.93 - 1.04)	0.60			0.99 (0.94 - 1.04)	0.65		
Booking SBP (mmHg) (5 unit increase)	86	**1.27 (1.08 - 1.50)**	**0.004**			**1.20 (1.05 - 1.37)**	**0.007**		
Booking DBP (mmHg) (5 unit increase)	86	**1.50 (1.17 - 1.90)**	**0.001**			**1.25 (1.05 - 1.49)**	**0.01**		
Booking MAP	86	**1.05 (1.01 - 1.08)**	**0.006**			**1.05 (1.01 - 1.08)**	**0.008**		
Ethnicity White vs not white	182	0.77 (0.39 - 1.53)	0.46			1.11 (0.61 - 2.01)	0.74		
Smoking status Smoking compared to not	125	1.27 (0.43 - 3.76)	0.67			1.41 (0.47 - 4.26)	0.55		
Parity	182	**0.47 (0.26 - 0.87)**	**0.02**			**0.47 (0.26 - 0.87)**	**0.02**		
Previous FDIU	82	0.51 (0.17 - 1.58)	0.25			**0.34 (0.12 - 0.94)**	**0.04**		
Previous NND	82	0.66 (0.23 - 1.86)	0.43			0.52 (0.21 - 1.29)	0.16		
Fetal biometry									
BPD (mm) 5 units	136	**0.50 (0.36 - 0.70)**	**< 0.001**	**0.29 (0.16 - 0.53)**	**< 0.001**	**0.67 (0.52 - 0.86)**	**0.002**	**0.42 (0.25 - 0.70)**	**< 0.001**
HC (mm) 5 units	168	**0.86 (0.80 - 0.92)**	**< 0.001**	**0.62 (0.51 - 0.76)**	**< 0.001**	**0.89 (0.84 - 0.94)**	**< 0.001**	**0.66 (0.56 - 0.79)**	**< 0.001**
AC (mm) 5 units	173	**0.84 (0.78 - 0.90)**	**< 0.001**	**0.76 (0.67 - 0.87)**	**< 0.001**	**0.86 (0.81 - 0.92)**	**< 0.001**	**0.76 (0.68 - 0.86)**	**< 0.001**
FL (mm) 5 units	170	**0.55 (0.42 - 0.72)**	**< 0.001**	**0.40 (0.25 - 0.64)**	**< 0.001**	**0.57 (0.49 - 0.73)**	**< 0.001**	**0.32 (0.20 - 0.52)**	**< 0.001**
EFW (g) (25 units)	170	**0.85 (0.80 - 0.91)**	**< 0.001**	**0.71 (0.62 - 0.81)**	**< 0.001**	**0.89 (0.85 - 0.93)**	**< 0.001**	**0.75 (0.67 - 0.83)**	**< 0.001**
EFW customised centile	147	**0.93 (0.86 - 1.00)**	**0.05**	**0.91 (0.84 - 0.99)**	**0.02**	**0.95 (0.91 - 1.00)**	**0.04**	**0.94 (0.90 - 0.99)**	**0.02**
EFW non-customised centile	170	**0.80 (0.68 - 0.96)**	**0.01**	**0.77 (0.64 - 0.92)**	**0.005**	**0.92 (0.86 - 0.98)**	**0.01**	**0.90 (0.84 - 0.97)**	**0.007**
Liquor volume									
Deepest pool (cm)	78	**0.53 (0.35 - 0.80)**	**0.007**	**0.47 (0.28 - 0.78)**	**0.003**	**0.62 (0.43 - 0.91)**	**0.02**	**0.60 (0.40 - 0.88)**	**0.01**

AFI (cm)	118	**0.84 (0.74 - 0.95)**	**0.006**	**0.79 (0.68 - 0.92)**	**0.02**	**0.87 (0.78 - 0.97)**	**0.01**	**0.85 (0.76 - 0.95)**	**0.004**
Fetal Dopplers									
UA PI (0.1 increment)	78	**1.16 (1.02 - 1.30)**	**0.02**	**1.17 (1.03 - 1.33)**	**0.01**	**1.16 (1.03 - 1.30)**	**0.02**	**1.17 (1.03 - 1.32)**	**0.02**
UA RI	69	**1.88 (1.14 - 3.09)**	**0.01**	**1.96 (1.16 - 3.30)**	**0.01**	**1.88 (1.15 - 3.07)**	**0.01**	**1.98 (1.18 - 3.33)**	**0.01**
UA EDF	154								
Present	60	Reference		Reference		Reference		Reference	
Absent	86	**8.99 (2.59 - 31.3)**	**0.001**	**12.4 (3.36 - 45.5)**	**< 0.001**	**6.59 (2.79 - 15.6)**	**< 0.001**	**8.45 (3.40 - 21.0)**	**< 0.001**
Reversed	8	**23.6 (3.54 - 157)**	**0.001**	**72.6 (8.22 - 641)**	**< 0.001**	**15 (2.47 - 91.0)**	**0.003**	**30.6 (4.36 - 215)**	**0.001**
Present/not present	60/94	**9.72 (2.82 - 33.5)**	**< 0.001**	**13.4 (3.70 - 48.6)**	**< 0.001**	**6.98 (2.98 - 16.4)**	**< 0.001**	**9.00 (3.65 - 22.2)**	**< 0.001**
MCA PI (0.1 increment)	71	0.90 (0.73 - 1.09)	0.27	0.86 (0.68 - 1.08)	0.20	0.83 (0.69 - 1.01)	0.06	**0.80 (0.65 - 0.99)**	**0.04**
MCA RI (0.1 increment)	62	0.57 (0.25 - 1.32)	0.19	0.52 (0.19 - 1.40)	0.20	**0.40 (0.17 - 0.92)**	**0.03**	**0.37 (0.15 - 0.94)**	**0.04**
DV PIV (0.1 increment)	45	0.97 (0.80 - 1.18)	0.78	0.92 (0.69 - 1.23)	0.59	1.00 (0.85 - 1.18)	0.99	0.98 (0.82 - 1.18)	0.84
DV PVIV (0.1 increment)	47	0.93 (0.74 - 1.18)	0.59	0.99 (0.72 - 1.34)	0.93	0.92 (0.75 - 1.13)	0.44	0.98 (0.76 - 1.25)	0.84
DV a-wave	74								
Present	65	Reference		Reference		Reference		Reference	
Absent	7	5.4 (0.91 - 32.3)	0.06	7.38 (0.93 - 58.6)	0.06	6.25 (0.69 - 56.6)	0.10	7.12 (0.67 - 75.5)	0.10
Reversed	2	2.71 (0.16 - 45.7)	0.49	0.88 (0.03 - 30.7)	0.95	-		-	
Normal	65	Reference		Reference		Reference		Reference	
Abnormal	9	**4.51 (0.97 - 20.9)**	**0.06**	4.49 (0.75 - 26.7)	0.10	**8.75 (1.02 - 73.4)**	**0.05**	8.60 (0.87 - 85.3)	0.07
Maternal Dopplers									
Mean UtA PI (0.1 increment)	126	1.06 (0.99 - 1.16)	0.10	**1.11 (1.00 - 1.22)**	**0.05**	1.01 (0.95 - 1.09)	0.68	1.03 (0.95 - 1.11)	0.49
Mean UtA RI (0.1 increment)	125	**1.78 (1.11 - 2.87)**	**0.02**	**2.05 (1.23 - 3.43)**	**0.006**	1.03 (0.73 - 1.47)	0.85	1.08 (0.74 - 1.56)	0.69
Log mean UtA PI	126	2.36 (0.66-8.47)	0.19	2.82 (0.68-11.70)	0.15	0.89 (0.31-2.55)	0.82	0.90 (0.30-2.76)	0.86
Log mean UtA RI	125	**32.24 (1.23-841.7)**	**0.04**	**42.27 (1.47-1217.7)**	**0.03**	0.67 (0.07-6.38)	0.73	0.63 (0.06-6.60)	0.70
Notching	140								
No notch	44	Reference		Reference		Reference		Reference	
Unilateral notch	28	2.24 (0.78 - 6.35)	0.13	**3.26 (1.02 - 10.5)**	**0.05**	2.37 (0.90 - 6.25)	0.08	**3.03 (1.06 - 8.63)**	**0.04**
Bilateral notch	68	1.22 (0.50 - 2.98)	0.67	1.81 (0.66 - 4.96)	0.25	1.34 (0.61 - 2.95)	0.47	1.72 (0.73 - 4.04)	0.21
Notch present	44	Reference		Reference		Reference		Reference	
No notch	96	1.48 (0.64 - 3.38)	0.36	2.19 (0.85 - 5.64)	0.10	1.59 (0.76 - 3.31)	0.22	2.03 (0.91 - 4.55)	0.09
Placental biometry									
Largest diameter (cm)	88	0.81 (0.61 - 1.07)	0.13	0.81 (0.61 - 1.07)	0.14	0.82 (0.64 - 1.05)	0.12	0.82 (0.64 - 1.05)	0.12
Smallest diameter (cm)	84	**0.70 (0.53 - 0.93)**	**0.02**	**0.72 (0.54 - 0.95)**	**0.02**	**0.76 (0.60 - 0.98)**	**0.03**	**0.77 (0.60 - 0.99)**	**0.04**
Depth (cm)	86	1.20 (0.85 - 1.70)	0.29	1.22 (0.86 - 1.73)	0.27	1.05 (0.77 - 1.45)	0.75	1.06 (0.77 - 1.46)	0.72

PEC ([placental diameter * placental width]/depth)	84	**0.95 (0.91 - 0.99)**	**0.01**	**0.95 (0.91 - 0.99)**	**0.01**	**0.97 (0.94 - 1.00)**	**0.03**	**0.97 (0.94 - 0.99)**	**0.03**
PSA (placental diameter * placental width)	84	**0.98 (0.97 - 1.00)**	**0.03**	**0.98 (0.97 - 1.00)**	**0.03**	**0.98 (0.97 - 0.99)**	**0.03**	**0.98 (0.97 - 0.99)**	**0.03**
Gestational age	182	**0.96 (0.94 - 0.98)**	**< 0.001**			**0.97 (0.96 - 0.99)**	**0.002**		
Blood pressure									
Systolic (mmHg) 5 unit increment	56	1.17 (0.96 - 1.45)	0.13	1.13 (0.87 - 1.46)	0.36	**1.29 (1.04 - 1.59)**	**0.02**	1.24 (0.98 - 1.57)	0.07
Diastolic (mmHg) 5 unit increment	56	1.11 (0.84 - 1.47)	0.45	1.09 (0.74 - 1.60)	0.66	**1.32 (0.99 - 1.75)**	**0.06**	1.35 (0.95 - 1.91)	0.09
Sex									
Male	99	Reference				Reference			
Female	69	0.84 (0.42-1.67)	0.62			0.71 (0.39-1.30)	0.26		

3.2.2.2. Multivariable analysis

Following univariable analysis, six candidate prognostic factors were identified, and taken forwards to be tested in different combinations in a multivariable model: gestational age at delivery, EFW, AFI, UA EDF, mean UtA RI and PSA. Factors selected for the prediction of overall death included: gestational age at delivery, EFW, AFI, UA EDF and PSA. All of the fetal biometry measurements were identified as potential candidate factors, but due to multicollinearity only one should be taken forwards. Despite being the product of a linear regression equation, EFW was selected as it represents all biometry measurements. Single deepest vertical pool (SDVP) and UA PI also had predictive ability, but there were fewer measurements recorded in the dataset than there were for AFI and UA EDF status respectively, so they were not selected to be tested in the final model.

3.2.2.2.1. Prediction of FDIU

Table 3.10 summarises the results of the backward selection to show how the final model was derived. Model 5 (gestation at diagnosis, EFW, UA EDF and mean UtA RI) was selected as the best performing model, although there was no significant difference between the AUC values for the five models (P = 0.28) (Figure 3.4). The number of events (FDIU) in the original cohort is 45, so by following the rule of thumb of ten events per candidate predictor parameter considered for inclusion (196), having four to five parameters in the final model is appropriate. Although a single variable, UA EDF is considered as two parameters as it has two categories (present vs. absent/reversed).

Table 3.10: Model coefficients, AUC and R^2 values for backwards model selection to predict FDIU at time of eFGR diagnosis. Model 5 (highlighted) was chosen as the best performing model.

Covariate	1		2		3		4		5	
	Coefficient (95% confidence interval)	**P value**	**Coefficient (95% confidence interval)**	**P value**	**Coefficient (95% confidence interval)**	**P value**	**Coefficient (95% confidence interval)**	**P value**	**Coefficient (95% confidence interval)**	**P value**
Gestational age (days)	0.02 (-0.11-0.16)	0.76	0.00 (–0.09-0.09)	0.94	0.04 (–0.05-0.13)	0.39	–0.03 (–0.10-0.05)	0.46	0.03 (–0.03-0.10)	0.32
EFW (g)	–0.01 (–0.02-0.01)	0.26	–0.01 (–0.02-0.001)	0.09	-0.01 (–0.02-0.00)	0.05	–0.01 (–0.01-0.00)	0.13	–0.01 (–0.02-0.00)	0.004
AFI (cm)	–0.11 (–0.36-0.14)	0.38	–0.11 (–0.30-0.09)	0.29			–0.13 (–0.30-0.05)	0.16		
UA EDF Present Absent/reversed	 Reference 1.98 (–0.59-4.55)	 0.13	 Reference 1.93 (0.12-3.75)	 0.04	 Reference 2.36 (0.13-4.59)	 0.04	 Reference 1.76 (0.24-3.28)	 0.02	 Reference 2.21 (0.57-3.84)	 0.008
Mean UtA RI	–3.80 (14.0-6.43)	0.47	1.27 (–5.23-7.77)	0.71	–0.96 (–8.25-6.33)	0.80			3.98 (–1.38-9.35)	0.15
PSA	–0.02 (–0.04-0.01)	0.28			–0.02 (–0.04-0.00)	0.07				
Constant	1.88 (–14.6-18.4)	0.82	0.16 (–12.1-12.4)	0.98	–3.28 (–15.3-8.78)	0.59	5.73 (–4.69-16.2)	0.28	–7.11 (–16.5-2.30)	0.14
AUC R^2	0.79 (0.70-0.89) 0.335		0.82 (0.73-0.91) 0.361		0.80 (0.71-0.89) 0.331		0.80 (0.71-0.89) 0.336		0.82 (0.74 -0.91) 0.364	

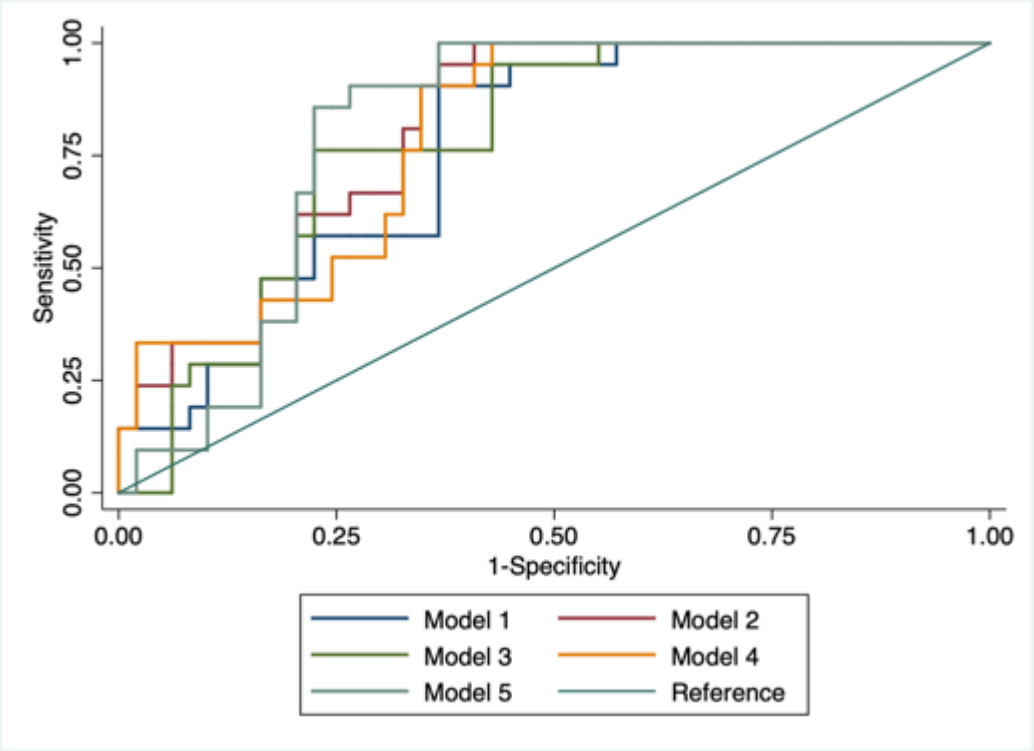


Figure 3.4: ROC curve for each of the five proposed models to predict FDIU.

The estimated model is summarised below (where UA EDF = 1 represents absent/reversed EDF):

$$Log\ odds\ of\ occurrence\ of\ FDIU$$
$$= \left(0.03 * gestational\ age\ at\ scan\ (days)\right) - \left(0.01 * EFW(g)\right)$$
$$+ (2.21 * UA\ EDF = 1) + (3.98 * Mean\ UtA\ RI) - 7.11$$

For a case diagnosed at 23^{+4} weeks, with an EFW of 390g, absent EDF and a mean UtA RI of 0.78 at diagnosis, the estimated probability of an FDIU occurring would be 51%:

$$p = \frac{\exp\left((0.03 * ((23 * 7) + 4) + (0.01 * 390) + (2.21 * 1) + (3.98 * 0.78) - 7.11\right)}{1 + \exp\left((0.03 * 165) + (0.01 * 390) + (2.21 * 1) + (3.98 * 0.78) - 7.11\right)} = 0.51$$

Instead of using the model to give an estimated probability of FDIU, a threshold can be applied, above which the likely outcome is taken to be FDIU. This gives a dichotomous live birth/FDIU outcome, which can be easier to interpret. To determine the optimum cut off value to distinguish between FDIU and live birth, a plot was first made of the sensitivity/specificity against probability cut off to investigate how this relationship changed (Figure 3.5).

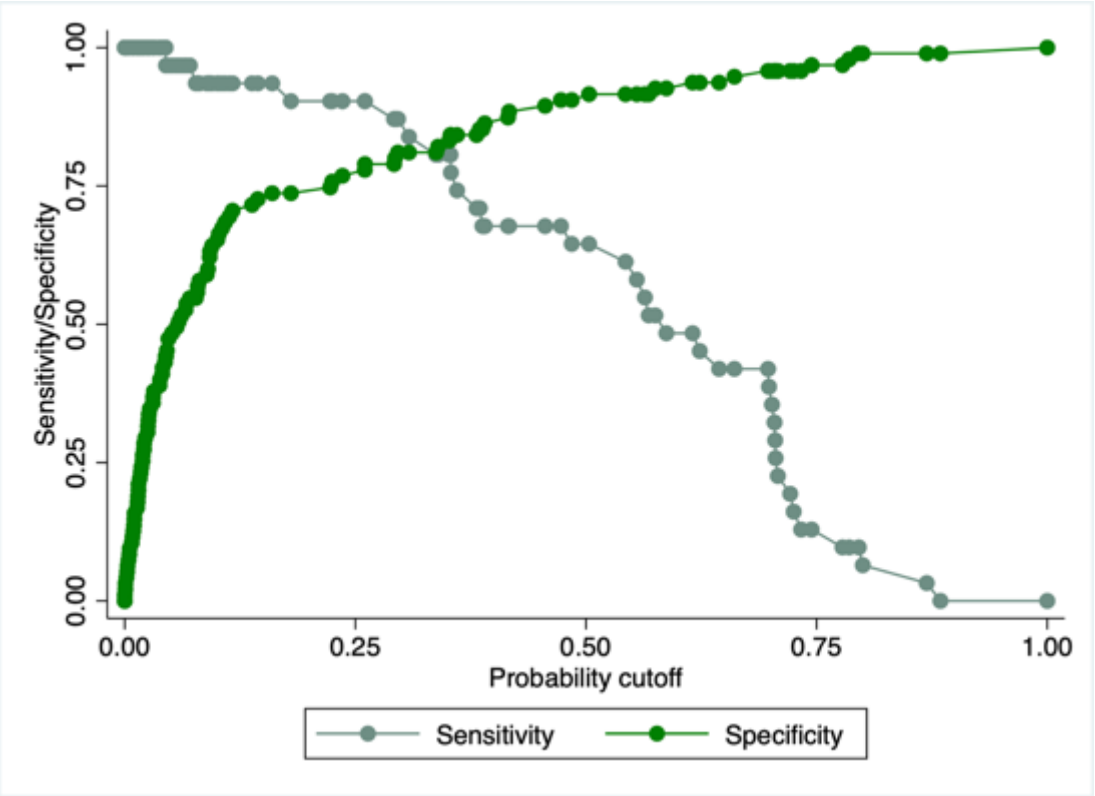


Figure 3.5: Plot of sensitivity/specificity against probability cut off for the FDIU model to determine the optimum probability cut off value to allow maximum sensitivity without compromising specificity.

The performance of the model was assessed at different cut off values, to determine the point at which it performed best, summarised by Table 3.11. A cut off of 0.25 was taken as the final cut off, in order to maximise sensitivity and negative predictive value. Above this value the outcome is taken to be FDIU, below this value the outcome is likely to be survival. A NPV of 96.1 means that if a negative result is obtained, clinicians can be confident that the pregnancy is likely to result in a live birth.

Table 3.11: Model classification according to probability cut off value.

Cut off value	**0.2**	**0.25**	**0.3**
Sensitivity (%)	90.3	90.3	83.9
Specificity (%)	74.7	77.9	81.1
Positive predictive value (%)	53.9	57.1	59.1
Negative predictive value (%)	96.0	96.1	93.9
+LR	3.6	4.1	4.4
-LR	0.13	0.12	0.20
Missed cases of FDIU	3	3	5

3.2.2.2.1.1. Logistic regression diagnostics

A link test was performed to assess model specification. The linear predicted value was statistically significant (P = 0.01) but the linear predicted value squared was not significant (P = 0.25), suggesting that the logit function was the correct function to use, all relevant variables are included and any significant additional predictors would only be found by chance.

The Hosmer and Lemeshow goodness of fit test suggests the model is well calibrated (P = 0.89), with the predicted and observed frequencies closely matching (using groups = 10).

The cohort was tested for influential observations. Figure 3.6 shows the Pearson's residuals (a-b), deviance residuals (c-d) and the leverage of each observation (e-f) plotted against predicted probabilities, and as an index plot. This highlights four cases with potential increased influence on the model, which are summarised in Table 3.12.

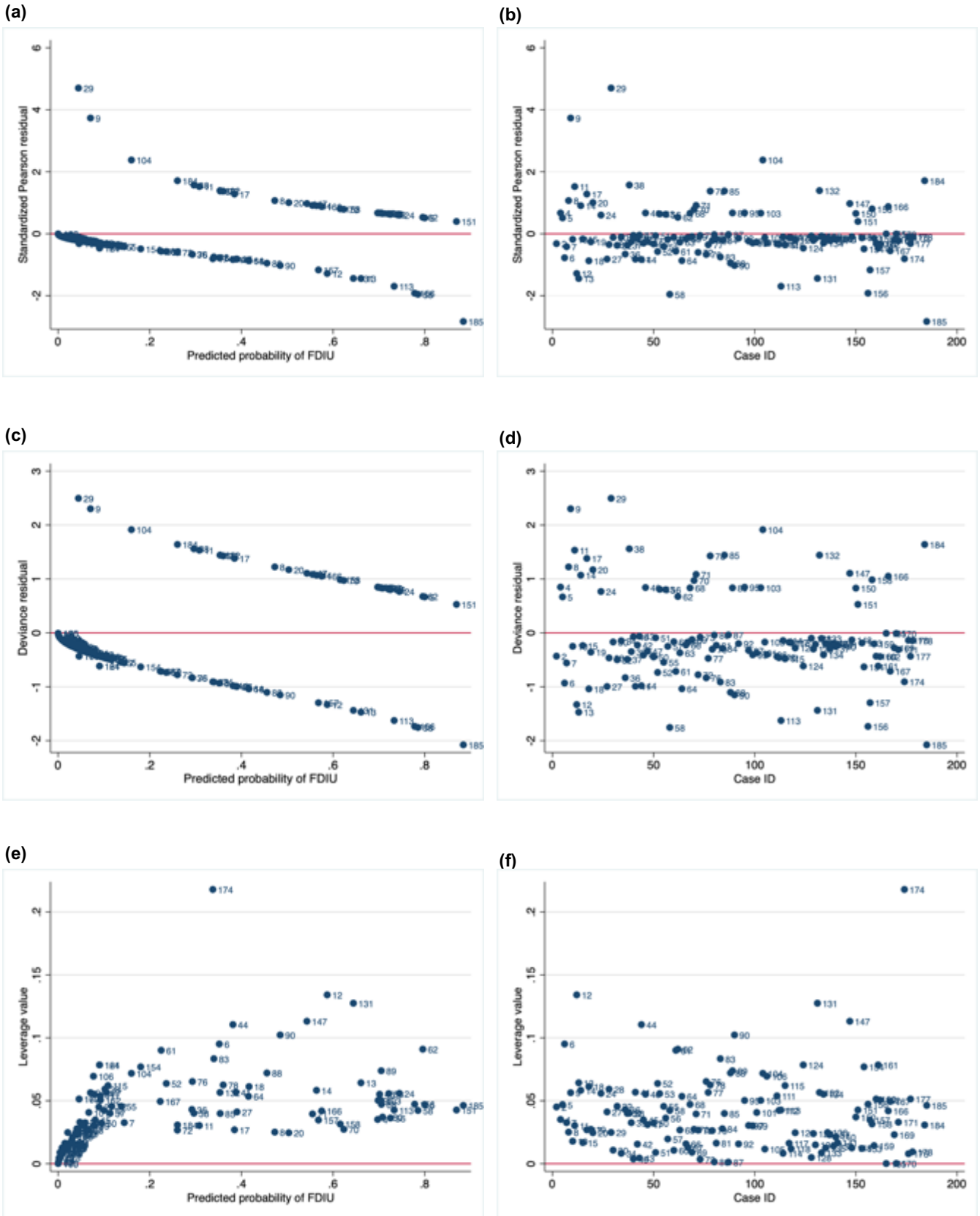


Figure 3.6: Pearson's residuals **(a-b**), deviance residuals **(c-d)** and the leverage of each observation **(e-f)** plotted against predicted probabilities (a, c, e), and as an index plot (b, d, f). This highlights four cases with potential increased influence on the model (case ID 9, 29, 104, 174).

Table 3.12: Summary of potential influential observations for prediction of FDIU.

Case ID	Gestational age at scan (weeks + days)	EFW at scan (g)	UA EDF	Mean UtA RI	Pregnancy outcome
9	25 + 3	423	Present	0.67	FDIU, 28 + 1, 460g
29	24 + 2	483	Present	0.77	FDIU, 27 + 3, 500g
104	29 + 2	682	Absent/reversed	0.77	FDIU, 30 + 1, 620g
174	23 + 2	246	Present	0.82	Live birth, 31 + 0, 467g

Case 9 and case 29 both had EDF present at the time of diagnosis but ended in FDIU. Case 104 was diagnosed at a much later gestation than the majority of cases, and the EFW was overestimated at the time of diagnosis (comparing EFW to the birthweight). Case 174 had a much lower than average EFW at the time of diagnosis but resulted in a live birth.
Removing these observations from the cohort and repeating the logistic regression does not significantly alter model performance (P = 0.30).

3.2.2.2.1.2. Internal validation/bootstrapping/optimism adjustment

Non-parametric bootstrapping was used as a method of internal validation to assess the potential overfitting of the model, using 500 samples with replacement from the original database. The bootstrap coefficients are summarised in Table 3.13.

Table 3.13: Bootstrap coefficients from interval validation of multivariable FDIU model.

Variable	Observed coefficient (95% confidence interval)	P value
Gestational age (days)	0.04 (–0.05-0.12)	0.41
EFW (g)	–0.01 (–0.02-0.00)	0.016
UA EDF Absent/reversed	 2.21 (0.63-3.78)	 0.006
Mean UtA RI	3.98 (–2.16-10.1)	0.21
Constant	–7.11 (–18.2-4.01)	0.21
	AUC: 0.885; R^2*:* 0.364	

Apparent and internal validation statistics of the final model are summarised in Table 3.14.
Following adjustment for optimism, the C-statistic (equal to the AUC) was found to be 0.897, with a CITL of -0.28 and a calibration slope of 0.91.

Table 3.14: Internal validation model diagnostics.

	C-statistic	Calibration in the large	Calibration slope
Apparent performance	0.902	–0.245	0.952
Internal validation test performance	0.905	–0.238	0.961
Average optimism	0.01	0.03	0.04
Optimism-corrected performance	0.897	–0.275	0.912

A uniform shrinkage factor of 0.961 (calibration slope of the internal validation performance) was applied to the beta-coefficients of the original model to give the final model, adjusted for overfitting:

$$\ln\left(\frac{p}{1-p}\right) = \left(0.0288 * gestational\ age\ at\ scan\ (days)\right) - \left(0.0096 * EFW(g)\right) + (2.124 * (UA\ EDF = 1) + (3.824 * Mean\ UtA\ RI) - 6.833$$

3.2.2.2.2. Prediction of overall death

Results of the backward variable selection are summarised in Table 3.15. Systolic BP at the time of scan and UtA notching were also considered as candidate variables, but due to the smaller numbers of cases with these data recorded at the time of diagnosis they were not included in the backwards selection, as this would have restricted the number of cases included in model development. Model 4 was selected as the final model. This did not have the highest R^2 value, however the coefficients were closer to significance in this model and allowing for fewer variables avoid potential overfitting. The AUC values for the four models are not significantly different from one another (P = 0.12) (Figure 3.7).

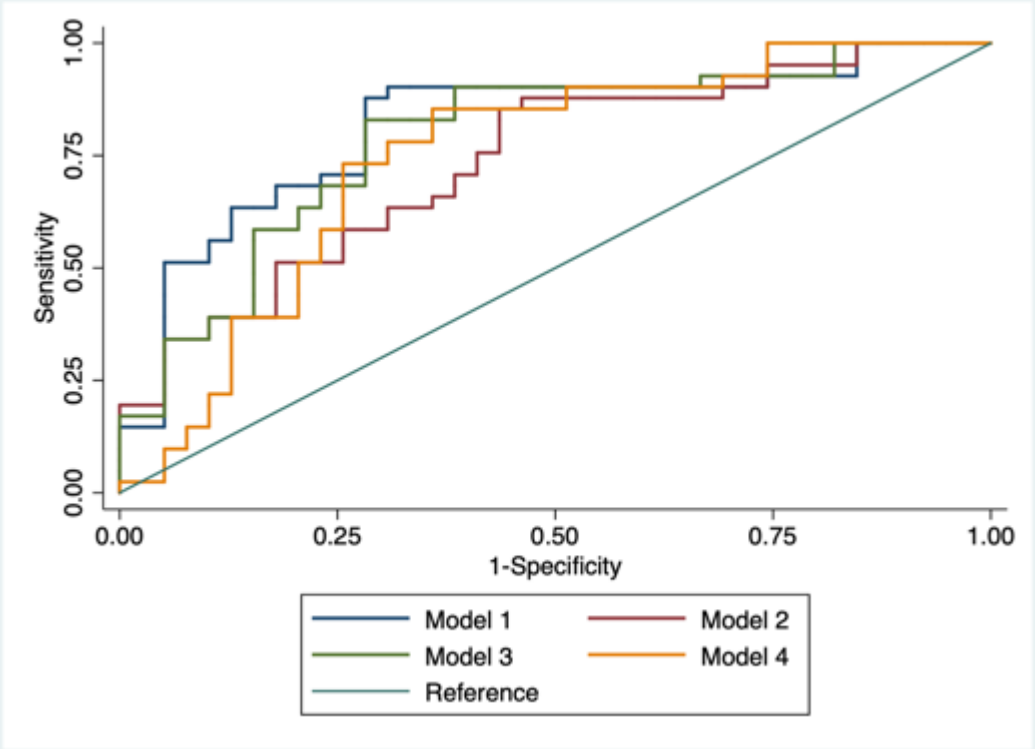


Figure 3.7: ROC curve for each of the proposed four models to predict overall death.

Table 3.15: Model coefficients, AUC and R^2 values for backwards model selection to predict overall death (FDIU or neonatal/infant death) at time of eFGR diagnosis. Model 4 (highlighted) was chosen as the multivariable model.

Covariate	1		2		3		4	
	Coefficient (95% confidence interval)	**P value**	**Coefficient (95% confidence interval)**	**P value**	**Coefficient (95% confidence interval)**	**P value**	**Coefficient (95% confidence interval)**	**P value**
Gestational age (days)	–0.05 (–0.17 - 0.06)	0.36	0.01 (–0.01 - 0.11)	0.88	–0.03 (–0.09 -0.04)	0.41	0.01 (–0.03 - 0.06)	0.56
EFW (g)	0.00 (–0.01 - 0.01)	0.70	–0.01 (–0.02 - 0.00)	0.12	0.00 (–0.01 - 0.00)	0.21	–0.01 (–0.01 - 0.00)	0.03
AFI (cm)	–0.17 (0.41 - 0.07)	0.16	–0.14 (–0.35 - 0.07)	0.20	–0.06 (–0.21 - 0.09)	0.40		
UA EDF Present Absent/reversed	 Reference 2.61 (–0.05 - 5.27)	 0.06			 Reference 1.70 (0.38 - 3.02)	 0.01	 Reference 1.29 (0.24 - 2.33)	 0.02
PSA	0.00 (–0.03 - 0.04)	0.81	–0.01 (–0.03 - 0.20)	0.66				
Constant	9.98 (–7.31 - 27.3)	0.26	4.99 (–11.0 - 21.0)	0.54	5.72 (–2.82 - 14.3)	0.19	–1.12 (–7.40 - 5.17)	0.73
AUC R^2	0.817 (0.72 - 0.93) 0.401		0.736 (0.63 - 0.84) 0.327		0.789 (0.69 - 0.89) 0.27		0.746 (0.63 - 0.86) 0.193	

The estimated model is summarised below:

$$Log\ odds\ of\ overall\ death = (0.01 * gestational\ age\ at\ scan\ (days)) - \left(0.01 * EFW(g)\right) + (1.29 * UA\ EDF = 1) - 1.12$$

A case diagnosed at 24^{+0} weeks' gestation, with an EFW of 503g and absent EDF would have an estimated 47% chance of death (either FDIU or neonatal death) occurring.

Figure 3.8 shows a plot of sensitivity/specificity against probability cut off. A value of 0.25 was chosen as the cut off above which the predicted outcome was taken to be death (FDIU or neonatal/infant death). Classification statistics for the cut off are summarised in Table 3.16.

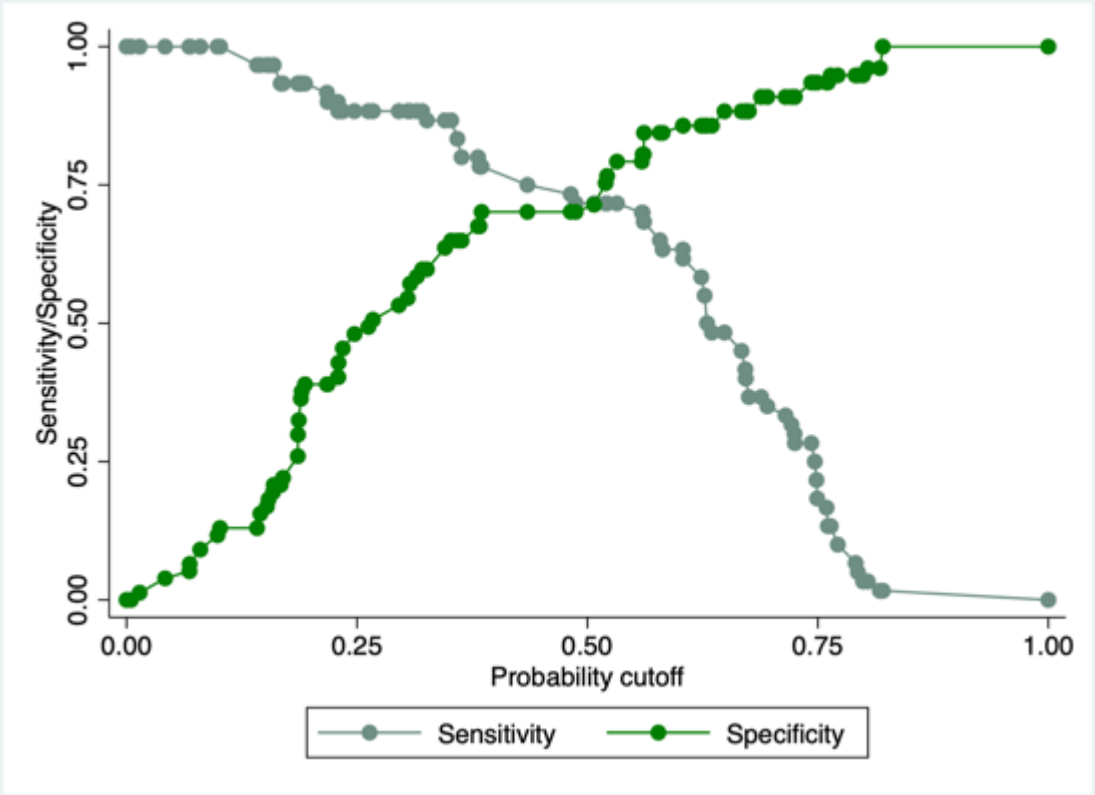


Figure 3.8: Plot of sensitivity/specificity against probability cut off for the overall death model to determine the optimum probability cut off value to allow maximum sensitivity without compromising specificity.

Table 3.16: Model classification according to probability cut off value

Cut off value	**0.2**	**0.25**	**0.4**
Sensitivity (%)	91.7	88.3	88.3
Specificity (%)	39.0	49.4	54.6
Positive predictive value (%)	53.9	57.6	60.2
Negative predictive value (%)	85.7	84.4	85.7
+LR	1.5	1.7	1.9
-LR	0.21	0.24	0.21
Missed cases of overall death	5	7	7

3.2.2.2.2.1. Logistic regression diagnostics

Performing a link test to assess model specification revealed the linear predicted value to be statistically significant (P < 0.001) and the linear predicted value squared to be not significant (P =

0.88). However, the Hosmer and Lemeshow goodness of fit test indicates inadequate fit in this instance (P = 0.002). This suggests that the observed event rates do not match the expected event rates. This information, together with the low R^2 value, the moderate AUC and the low +/-LR values suggest that this model will not perform as well as the model developed to predict FDIU, potentially due to differences in the pregnancies that ended in FDIU compared to the neonatal/infant death subcohorts. For this reason, a model to predict overall death at the time of diagnosis was not taken forward for further development, and the remainder of the logistic regression diagnostics are not included in this thesis.

3.3.2.3. Predictive factors - Gestational age at delivery

These data are summarised in Table 3.17. None of the maternal booking characteristics were predictive of the gestational age at delivery. Fetal biometry measurements were predictive of gestational age at delivery, however when adjusted for the gestational age at the time of diagnosis, only HC and AC were significantly associated. A 5mm increase in HC was associated with prolonged pregnancy by 1.13 (0.07-2.20; P < 0.001) days, and a 5mm increase in AC was associated with a 0.73 (0.01-1.44; P < 0.001) day increase in gestation. EFW and both customised/non-customised centile were associated with gestation, with a greater weight/centile leading to a later gestation at delivery. Liquor volume showed a similar relationship; the higher the liquor volume at diagnosis, the greater the gestational age at delivery.

In terms of umbilical artery Doppler, the higher the PI and RI at diagnosis, the earlier the gestation at delivery, with every 0.1 increase in UA PI conferring a 1.22 day reduction in the gestation at delivery (-1.95- -0.48; p = 0.001). In terms of UA EDF, if this was absent or reversed at presentation, delivery on average took place almost 2 weeks earlier than if it was present (-12.34 days (-17.38- -7.31; P < 0.001). Adjusting for gestation at the time of recording of the UA parameters had no appreciable effect on the coefficients.

Maternal UtA RI, but not PI, was significantly associated with gestation at delivery, with a 0.1 increase in RI associated with a 2.72 day earlier delivery (-5.47- -0.04; P = 0.05); again this remained the same when adjusted for gestational age at diagnosis. The presence of a notch at diagnosis was associated with 8 days less gestation at delivery, which persisted when adjusted for gestation (-8.23 (-13.86- -2.61); P = 0.004).

Placental diameter, but not depth, was associated with gestational age at delivery; the larger the placental diameter, the later the gestation at delivery.

Maternal BP was significantly associated with gestational age at delivery, with an increasing BP associated with a decreasing gestation, but this relationship did not remain significant when the gestational age at the time of BP measurement was adjusted for.

Finally, no relationship was found between infant sex and gestational age at delivery.

Table 3.17: Univariable analysis to determine what factors at the time of diagnosis are associated with gestational age at delivery. Significant associations (P < 0.05) are highlighted in bold.

Variable		Univariable analysis		Gestation-adjusted	
		Coefficient (95% confidence interval)	P value	Coefficient (95% confidence interval)	P value
Age	182	–0.11 (–0.53 - 0.30)	0.60		
Booking weight (kg)	147	–0.06 (–0.22 - 0.1)	0.43		
Booking height (cm)	148	–0.20 (–0.61 - 0.22)	0.35		
BMI (kg/m^2)	147	–0.10 (–0.54 - 0.33)	0.64		
Booking SBP (mmHg) (5 unit increase)	86	**–1.13 (–2.20 - –0.06)**	**0.04**		
Booking DBP (mmHg) (5 unit increase)	86	**–2.01 (–3.39 - –0.64)**	**0.01**		
Booking MAP	86	**–0.33 (–0.58 - –0.08)**	**0.01**		
Ethnicity White vs not white	182	2.14 (–2.78 - 7.05)	0.39		
Smoking status Smoking compared to not	125	–2.89 (–11.48 - 5.71)	0.51		
Multiparous	182	2.34 (–2.63 - 7.31)	0.35		
Previous FDIU	82	8.75 (–0.43 - 17.9)	0.06		
Previous NND	82	**11.3 (2.07 - 20.55)**	**0.02**		
Fetal biometry					
BPD (mm) 5 units	136	**2.67 (0.81 - 4.53)**	**0.01**	**3.27 (–0.31 - –6.84)**	**0.07**
HC (mm) 5 units	168	**0.83 (0.40 - 1.26)**	**< 0.001**	**1.13 (0.07 - 2.20)**	**0.04**
AC (mm) 5 units	173	**0.83 (0.40 - 1.26)**	**< 0.001**	**0.73 (0.01 - 1.44)**	**0.05**
FL (mm) 5 units	170	**2.65 (0.96 - 4.33)**	**0.002**	1.49 (–1.45 - 4.44)	0.32
EFW (g) (25 units)	170	**0.58 (0.32 - 0.85)**	**< 0.001**	**0.74 (0.23 - 1.25)**	**0.01**
EFW customised centile	147	0.21 (–0.02 - 0.44)	0.08	**0.28 (0.05 - 0.51)**	**0.02**
EFW non-customised centile	170	**0.27 (0.02 - 0.51)**	**0.04**	**0.32 (0.08 - 0.56)**	**0.01**
Liquor volume					
Deepest pool (cm)	78	2.38 (–0.47 - 5.23)	0.10	2.45 (–0.28 - 5.17)	0.08
AFI (cm)	118	**1.00 (0.27 - 1.73)**	**0.01**	**1.06 (0.35 - 1.77)**	**0.004**
Fetal Dopplers					
UA PI (0.1 increment)	78	**–1.22 (–1.95 - –0.48)**	**0.001**	**–1.21 (–1.90 - –0.52)**	**0.001**
UA RI (0.1 increment)	69	**–5.50 (–8.26 - –2.75)**	**< 0.001**	**–5.43 (–8.09 - –2.76)**	**< 0.001**

UA EDF	154				
Present	60	**Reference**		**Reference**	
Absent	86	**–11.10 (–16.13 - –6.07)**	**< 0.001**	**–11.85 (–16.63 - –7.07)**	**< 0.001**
Reversed	8	**–25.69 (–36.94 - –14.42)**	**< 0.001**	**–27.48 (–38.19 - –16.78)**	**< 0.001**
Present/not present	60/94	**–12.34 (–17.38 - –7.31)**	**< 0.001**	**–13.16 (–17.97 - –8.33)**	**< 0.001**
MCA PI (0.1 increment)	71	**1.42 (0.10 - 2.74)**	**0.04**	**1.31 (0.12 - 2.50)**	**0.03**
MCA RI (0.1 increment)	62	**7.71 (2.16 - 13.26)**	**0.01**	**6.26 (1.41 - 11.11)**	**0.01**
DV PIV (0.1 increment)	45	0.54 (–0.86 - 1.95)	0.44	0.67 (–0.65 - 1.99)	0.31
DV PVIV (0.1 increment)	47	0.16 (–1.47 - 1.80)	0.84	–0.24 (–1.73 - 1.25)	0.75
DV a–wave	74				
Present	65	Reference		Reference	
Absent	7	**–16.13 (–27.95 - –4.30)**	**0.008**	**–14.00 (–25.21 - –2.79)**	**0.02**
Reversed	2	15.01 (–6.32 - 36.35)	0.17	**20.52 (0.14 - 40.90)**	**0.05**
Normal/abnormal	65/9	–7.41 (–17.72 - 2.90)	0.16	–4.30 (–13.54 - 4.95)	0.36
Maternal Dopplers					
Mean UtA PI (0.1 increment)	126	–0.28 (–0.84 - 0.28)	0.33	–0.30 (–0.85 - 0.24)	0.27
Mean UtA RI (0.1 increment)	125	**–0.72 (–5.47 - –0.04)**	**0.05**	**–2.79 (–5.50 - –0.08)**	**0.04**
Log mean UtA PI	126	–4.69 (–13.32 - 3.94)	0.28	–5.04 (–13.45 - 3.36)	0.24
Log mean UtA RI	125	–17.44 (–35.55 - 0.67)	0.06	**–17.52 (–35.23 -0.20)**	**0.05**
Notching	140				
No notch	44	**Reference**		**Reference**	
Unilateral notch	28	**–8.22 (–16.22 - –0.21)**	**0.04**	**–7.74 (–15.29 - –0.19)**	**0.05**
Bilateral notch	68	**–8.15 (–14.56 - –1.74)**	**0.01**	**–8.58 (–14.62 - –2.54)**	**0.01**
No notching	44	**Reference**		**Reference**	
Unilateral/bilateral notching	96	**–8.00 (–13.95 - –2.04)**	**0.01**	**–8.23 (–13.86 - –2.61)**	**0.004**
Placental biometry					
Largest diameter	88	**2.28 (0.46 - 4.10)**	**0.02**	**2.37 (0.57 - 4.16)**	**0.01**
Smallest diameter	84	**2.45 (0.80 - 4.10)**	**0.004**	**2.33 (0.70 - 3.96)**	**0.01**
Depth	86	0.41 (–2.99 - 2.16)	0.75	–0.64 –-3.18 - 1.91)	0.62
PEC ([placental diameter * placental width]/depth)	84	**0.25 (0.07 - 0.43)**	**0.01**	**0.26 (0.08 - 0.43)**	**0.004**
PSA (placental diameter * placental width)	84	**0.15 (0.06 - 0.25)**	**0.002**	**0.16 (0.06 - 0.25)**	**0.002**
Gestational age (weeks)	182	**0.27 (0.14 - 0.40)**	**< 0.001**		
Blood pressure					
Systolic (mmHg) 5 increment	56	**–1.57 (–3.05 - –0.10)**	**0.04**	–0.97 (–2.45 - 0.51)	0.20

Diastolic (mmHg) 5 increment	56	**–2.24 (–4.41 - –0.06)**	**0.04**	–1.35 (–3.54 - 0.84)	0.22
Gender					
Male	182	Reference			
Female		4.11 (–0.85 - 9.08)	0.10		

3.3.2.4. Predictive factors: Birthweight

The pattern of significant predictive factors for birthweight was very similar to that of gestational age at delivery. These data are summarised in Table 3.18. All the fetal biometry measurements, EFW and EFW centile were predictive of birthweight, with every 25g increase in EFW at the time of diagnosis associated with a 40g higher birthweight at delivery (gestation-adjusted, 39.69g (32.60-46.78); P < 0.001). A 1cm increase in AFI conferred an increase in birthweight of almost 40g (gestation-adjusted, 38.80g (26.38-51.21); P < 0.001).

In terms of fetal Dopplers, every 0.1 increment in UA PI was associated with a 31.44g reduction in final birthweight (-45.50- -17.38; P < 0.001), and if UA EDF was absent or reversed at diagnosis, final birthweight was on average 319g less than if it was present (-402.69 – -235.7; P < 0.001).

Interestingly, maternal UtA PI, not RI, was predictive of birthweight, with each 0.1 increase in PI associated with a 114g reduction in birthweight (-303.20 – -7.00; P = 0.04). UtA notching showed a similar pattern to gestation at delivery, with the presence of a notch associated with a 150g reduction in birthweight (-154.03 (-253.14 – -54.92); P = 0.003).

In terms of placental biometry, the larger the placental diameter the greater the birthweight, but there was no relationship between placental depth and birthweight. Maternal BP at the time of diagnosis did not predict final birthweight, whether this was adjusted for gestation or not. Finally, infant sex was not found to be a significant predictor of final birthweight.

Table 3.18: Univariable analysis to determine what factors at the time of diagnosis are associated with birthweight. Significant associations (P < 0.05) are highlighted in bold.

Variable		Univariable analysis		Gestation-adjusted	
		Coefficient (95% confidence interval)	P value	Coefficient (95% confidence interval)	P value
Age	182	1.77 (–6.07 - 9.61)	0.66		
Booking weight (kg)	147	1.60 (–1.47 - 4.67)	0.31		
Booking height (cm)	148	–1.05 (–9.19 - 7.09)	0.80		
BMI (kg/m^2)	147	5.09 (–3.36 - 13.53)	0.24		
Booking SBP (mmHg) (5 unit increase)	86	–10.93 (–31.78 - 9.92)	0.30		
Booking DBP (mmHg) (5 unit increase)	86	–18.05 (–45.93 - 9.82)	0.20		
Booking MAP	86	–2.67 (–7.73 - 2.38)	0.29		
Ethnicity	182				
White vs not white		31.96 (–60.58 - 124.51)	0.50		
Smoking status	125				
Smoking compared to not		–23.21 (–194.64 - 148.22)	0.79		
Multiparous	182	**111.89 (18.96 - 204.82)**	**0.02**		
Previous FDIU	82	**360.94 (198.41 - 523.48)**	**< 0.001**		
Previous NND	82	**318.11 (146.52 - 489.71)**	**< 0.001**		
Fetal biometry					
BPD (mm) 5 units	136	**95.00 (64.50 - 125.50)**	**0.001**	**182.99 (127.29 - 238.68)**	**< 0.001**
HC (mm) 5 units	168	**28.27 (21.17 - 35.37)**	**< 0.001**	**69.39 (53.23 - 85.55)**	**< 0.001**
AC (mm) 5 units	173	**34.17 (27.70 - 40.64)**	**< 0.001**	**53.78 (43.68 - 63.88)**	**< 0.001**
FL (mm) 5 units	170	**115.78 (89.43 - 142.13)**	**< 0.001**	**178.72 (134.04 - 223.39)**	**< 0.001**
EFW (g) (25 units)	170	**21.05 (16.97 - 25.13)**	**< 0.001**	**39.69 (32.60 - 46.78)**	**< 0.001**
EFW customised centile	147	**11.41 (7.40 - 15.41)**	**0.001**	**13.38 (9.77 - 16.99)**	**< 0.001**
EFW non-customised centile	170	**13.33 (9.15 - 17.51)**	**< 0.001**	**14.81 (11.06 - 18.57)**	**< 0.001**
Liquor volume					
Deepest pool (cm)	78	**103.23 (52.59 - 153.88)**	**0.001**	**103.98 (56.30 - 151.66)**	**0.001**
AFI (cm)	118	**37.25 (23.87 - 50.62)**	**< 0.001**	**38.80 (26.38 - 51.21)**	**< 0.001**
Fetal Dopplers					
UA PI (0.1 increment)	78	**–31.55 (–46.46 - –16.64)**	**< 0.001**	**–31.44 (–45.50 - –17.38)**	**< 0.001**
UA RI (0.1 increment)	69	**–128.92 (–183.66 - –74.18)**	**< 0.001**	**–127.71 (–181.53 - 73.88)**	**< 0.001**
UA EDF	154				

Present	60	**Reference**		**Reference**	
Absent	86	**–293.11 (–386.13 - –200.08)**	**< 0.001**	**–311.20 (–395.84 - –226.56)**	**< 0.001**
Reversed	8	**–355.40 (–576.28 - –134.53)**	**0.002**	**–422.13 (–623.86 - –220.39)**	**< 0.001**
Present/not present	60/94	**–297.79 (–389.16 - –206.43)**	**< 0.001**	**–319.20 (–402.69 - –235.71)**	**< 0.001**
MCA PI (0.1 increment)	71	**22.31 (0.60 - 44.01)**	**0.04**	**19.67 (3.35 - 35.99)**	**0.02**
MCA RI (0.1 increment)	62	**111.83 (16.39 - 207.26)**	**0.02**	**80.29 (6.30 - 154.28)**	**0.03**
DV PIV (0.1 increment)	45	–13.45 (-36.35 - 9.45)	0.24	–11.44 (–33.03 - 10.15)	0.29
DV PVIV (0.1 increment)	47	–4.67 (–32.19 - –22.85)	0.73	–11.32 (–36.62 - 13.99)	0.37
DV a-wave	74				
Present	65	Reference		Reference	
Absent	7	–142.32 (–360.69 - 76.05)	0.20	–114.42 (–303.20 - 74.35)	0.23
Reversed	2	–309.65 (–677.07 - 57.76	0.10	–171.70 (–493.51 - 150.1)	0.29
Maternal Dopplers					
Mean UtA PI (0.1 increment)	126	–104.28 (–212.41 - 3.84)	0.06	**–109.01 (–211.03 - –7.00)**	**0.04**
Mean UtA RI (0.1 increment)	125	–470.05 (–1011.80 - 71.70)	0.09	–476.96 (–996.77 - 42.84)	0.07
Log mean UtA PI	126	–142.31 (–307.88 - 23.27)	0.09	–152.84 (–309.08 - 3.41)	0.06
Log mean UtA RI	125	–286.87 (–636.36 - 61.62)	0.11	–290.42 (–624.86 - 44.01)	0.09
Notching	140				
No notch	44	**Reference**		**Reference**	
Unilateral notch	28	**–221.87 (–360.64 - 83.11)**	**0.002**	**–209.39 (–338.17 - 80.61)**	**0.002**
Bilateral notch	68	**–129.38 (–241.03 - 17.72)**	**0.02**	**–136.16 (–239.74 - –32.58)**	**0.01**
No notching	44	**Reference**		**Reference**	
Unilateral/bilateral notching	96	**–152.41 (–259.29 - –45.54)**	**0.006**	**–154.03 (–253.14 - –-54.92)**	**0.003**
Placental biometry					
Largest diameter	88	**66.85 (35.82 - 97.87)**	**< 0.001**	**68.54 (37.91 - 99.16)**	**< 0.001**
Smallest diameter	84	**62.66 (34.28 - 91.07)**	**< 0.001**	**61.22 (32.87 - 89.58)**	**< 0.001**
Depth	86	–27.51 (–74.23 - 19.21)	0.25	–30.61 (–77.06 - 15.84)	0.19
PEC ([placental diameter * placental width]/depth)	84	**6.81 (3.78 - 9.84)**	**< 0.001**	**7.01 (4.03 - 9.98)**	**< 0.001**
PSA (placental diameter * placental width)	84	**4.20 (2.56 - 5.84)**	**< 0.001**	**4.23 (2.61 - 5.84)**	**< 0.001**
Gestational age (weeks)	182	**6.52 (4.07 - 8.97)**	**< 0.001**		
Blood pressure					
Systolic 5 increment (mmHg)	56	–23.11 (–51.06 - 4.83)	0.10	–11.11 (–39.01 - 16.78)	0.43
Diastolic (mmHg) 5 increment	56	–28.24 (–69.75 - 13.28)	0.18	–9.98 (–51.20 - 31.25)	0.63
Gender					
Female compared to male	182	43.08 (–51.22 - 137.39)	0.37		

3.3.3. Longitudinal data

Data acquired from across gestation have been analysed in two different ways. Firstly, serial ultrasound biometry measurements have been compared to determine if growth trajectory is prognostic of pregnancy outcome. Secondly, Doppler data have been used in a similar comparison to determine if the pattern of change of UtA and UA Doppler parameters are predictive of outcome.

3.3.1. Longitudinal weight change

Sequential ultrasound fetal biometry measurements can be used to determine if there is an altered rate of growth in eFGR pregnancies ending in FDIU or death. If the growth rate in these two cohorts is significantly different, then growth rate could be used as a prognostic factor in eFGR pregnancies.

For this analysis, the previously described data from St Mary's Hospital were combined with growth scan data from 30 eFGR cases managed at East Lancashire Hospitals NHS Trust between January 2017 and August 2019. Within this cohort, outcomes were as follows: 66.67% live birth, survived to discharge (n = 20), 23.33% FDIU (n = 7), 10% NND (n = 3).

In total, 211 eFGR cases had growth scans recorded, and a total of 1144 scans were included in this analysis. Cases had a median of 4 growth scans (range: 1-10).

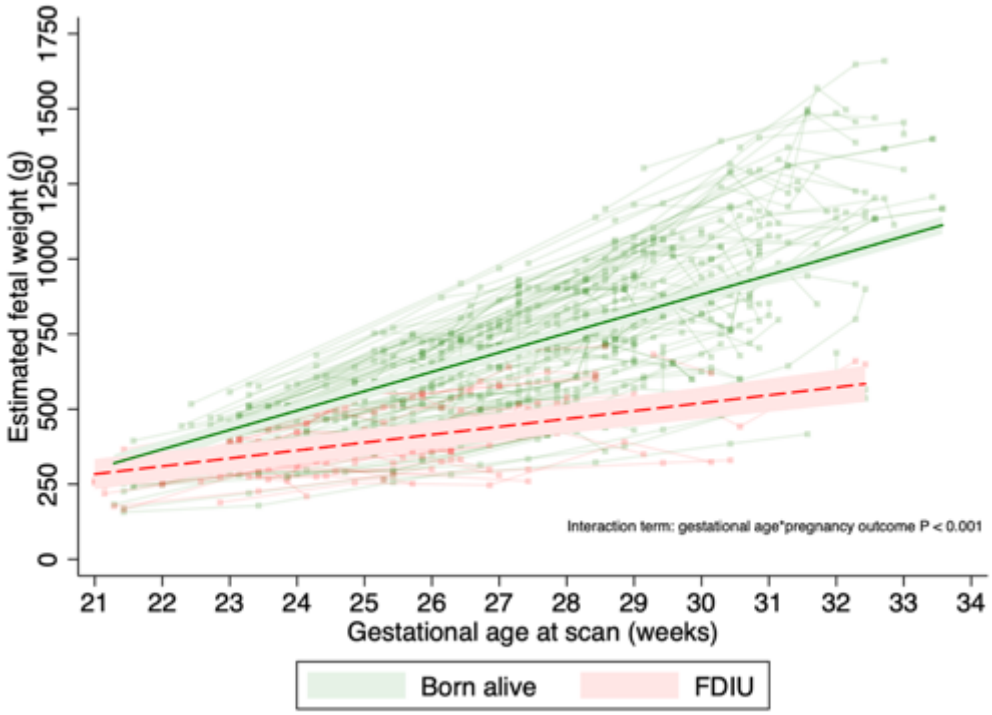
Table 3.19 summarises the results of the regression analysis for models (to predict FDIU, and to predict overall death (FDIU/NND/infant death). This suggests that longitudinal growth is adequately modelled using linear regression, as higher order polynomial terms were not significant. The interaction term between gestational age and pregnancy outcome was statistically significant for both FDIU and overall death, indicating that the growth rate varied depending on the pregnancy outcome, with those pregnancies ending in either FDIU or death having a slower rate of growth throughout gestation than those that end in live birth or survival. This suggests that weight gain can be used as a predictive factor. The analysis produced similar results when performed on raw data and log-transformed data; results of the raw data analysis are presented here for ease of interpretation in a clinical context.

Table 3.19: Mixed level regression to investigate longitudinal growth as a prognostic factor for both FDIU and overall death in eFGR cohort. The models highlighted in grey were chosen as those best fitted to the data.

Variable	FDIU		Overall death	
	Coefficient (95% confidence interval)	P value	Coefficient (95% confidence interval)	P value
LINEAR REGRESSION				
Gestational age (days)	8.63 (8.31-8.95)	< 0.001	8.63 (8.31-8.94)	< 0.001
Pregnancy outcome	–194.12 (–247.35 - –140.89)	< 0.001	–204.48 (–249.15 - –159.81)	< 0.001
Constant	–932.07 (–999.56 - –864.59)	< 0.001	–902.38 (–970.50 - –834.27)	< 0.001
Outcome included as interaction term				
Gestational age (days)	9.38 (9.05 - 9.70)	< 0.001	10.06 (9.72 - 10.40)	< 0.001
Pregnancy outcome	621.23 (467.32 - 775.13)	< 0.001	693.78 (569.83 - 817.65)	< 0.001
Pregnancy outcome*gestational age (days)	–4.52 (–5.32 - –3.72)	< 0.001	–4.81 (–5.43 - 4.19)	< 0.001
Constant	–1079.07 (–1148.44 –1009.70)	< 0.001	–1185.09 (–1257.49 - –1112.68)	< 0.001
POLYNOMIAL - QUADRATIC				
Gestational age (days)	11.02 (–15.17- –6.88)	< 0.001	–10.80 (–14.94 - –6.66)	< 0.001
Gestational age (days)^2	0.05 (0.04 - 0.06)	< 0.001	0.05 (-0.04 - 0.06)	< 0.001
Pregnancy outcome	–194.30 (–245.86 - –142.75)	< 0.001	–200.84 (–244.29 - –157.39)	< 0.001
Constant	893.02 (503.74 - 1282.31)	< 0.001	900.60 (512.12 - 1289.07)	< 0.001
Outcome included as interaction term				
Gestational age (days)	–5.47 (–10.17 - –0.78)	0.02	5.82 (–10.63 - –1.01)	0.02
Gestational age (days)^2	0.04 (0.03 - 0.05)	< 0.001	0.04 (0.03 - 0.05)	< 0.001
Pregnancy outcome	50.07 (–894.72 - 994.87)	0.92	–390.04 (–1162.91 - 382.83)	0.323
Pregnancy outcome*gestational age (days)	1.00 (–9.45 - 11.46)	0.85	6.52 (–1.84 - 14.88)	0.13
Pregnancy outcome*gestational age (days)^2	–0.01 (–0.04 - 0.20)	0.38	0.03 (–0.05 - –0.01)	0.01
Constant	315.38 (–129.75 - 760.51)	0.17	309.50 (–147.52 - 766.53)	0.18

Figure 3.9 shows the growth trajectory with actual values (shaded line graph) and predicted values with 95% confidence interval (line) to predict (a) live birth and (b) overall survival.

(a)



(b)

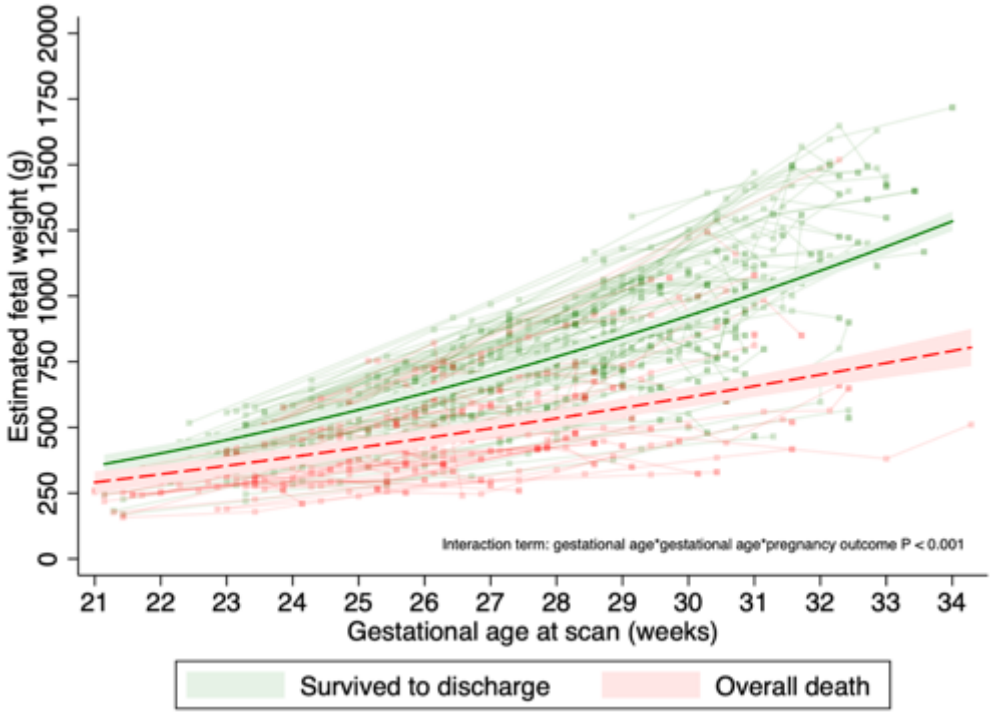


Figure 3.9: Growth trajectory according to pregnancy outcome. Shaded lines represent growth trajectory of each individual case. Solid lines represent prediction from regression analysis and 95% confidence interval. Plots are displayed according to pregnancy outcome, **(a)** Live birth compared to FDIU; **(b)** Survival to discharge compared to overall death.

3.3.1.2. Weight gain as a prognostic factor

The number of growth scans in each gestational age group is summarised by Table 3.20.

Table 3.20: Gestational age distribution of growth scans performed in the eFGR cohort.

Gestational age (weeks)	Number of growth scans	Percentage of scans performed (%)
<24	163	14.25
$24^{+0} – 25^{+6}$	188	16.43
$26^{+0} – 27^{+6}$	263	22.99
$28^{+0} – 29^{+6}$	264	23.08
≥30	266	23.25

Using the calculated figure for the weight gain for each case over each gestational age group, logistic regression was used to determine in which gestational age groups growth rate was significantly related to pregnancy outcome. In addition, the relationship was also explored using a standard fixed growth rate of 50g per week (100g over two weeks) (Table 3.21).

Table 3.21: Logistic regression to investigate 50g weekly weight gain as a prognostic factor during each gestational age timeframe. Significant associations are highlighted in grey.

	FDIU			Death		
Variable	**Odds ratio**	**P value**	**95% confidence interval**	**Odds ratio**	**P value**	**95% confidence interval**
<24 weeks						
Weight gain	0.65	0.43	0.22-1.90	0.45	0.29	0.10-2.00
Constant	1.49	0.43	0.55-4.07	3.86	0.05	0.98-15.19
$24^{+0} – 25^{+6}$ weeks						
Weight gain	0.47	0.005	0.28-0.80	0.26	< 0.001	0.13-0.51
Constant	0.88	0.65	0.50-1.54	3.08	0.003	1.48-6.37
$26^{+0} – 27^{+6}$ weeks						
Weight gain	0.55	0.003	0.38-0.81	0.59	0.003	0.42-0.83
Constant	0.55	0.02	0.33-0.90	1.13	0.60	0.70-1.85
$28^{+0} – 29^{+6}$ weeks						
Weight gain	1.01	0.94	0.83-1.22	0.90	0.26	0.75-1.08
Constant	0.12	< 0.001	0.07-0.23	0.40	< 0.001	0.25-0.62
≥30 weeks						
Weight gain	0.99	0.20	0.77-1.26	0.91	0.33	0.76-1.10
Constant	0.07	< 0.001	0.03-0.17	0.21	< 0.001	0.12-0.3700.

This highlights that growth rate is predictive of outcome between $24^{+0} – 25^{+6}$ weeks and $26^{+0} – 27^{+6}$ weeks with a 50g increase in weight being associated with a 53% reduction in the risk of FDIU between $24^{+0} – 25^{+6}$ weeks (OR 0.47 (0.28-0.80); P = 0.005) and a 45% reduction between $26^{+0} – 27^{+6}$ (OR 0.55 (0.38-0.81); P = 0.003). In terms of overall death, this translated to a 74% reduction in the risk between $24^{+0} – 25^{+6}$ weeks (OR 0.26 (0.13-0.51); P < 0.001) and a 41% reduction between $26^{+0} – 27^{+6}$ (OR 0.59 (0.42-0.83); P = 0.003).

3.3.2. Longitudinal uterine artery Doppler change

A total of 797 mean UtA PI measurements were taken from 126 eFGR cases (outcomes as follows: 61% live birth, survived to discharge/24% FDIU/15% neonatal/infant death). Each case had a median of 4 UtA Dopplers taken during pregnancy (range 1-19), with a median across all gestations of 1.46 (range 0.47-4.14). Logarithmic transformation of the UtA PI values did not change the outcome of the regression modelling, so values were kept as raw data.

3.3.2.1. Prediction of FDIU

In those cases that ended in a live birth, there was a linear decrease in mean UtA PI across gestation (Table 3.22, **Error! Reference source not found.**10). In those cases ending in FDIU, there was a more pronounced decrease across gestation, with the mean UtA PI higher earlier in gestation, and lower later in gestation. This difference in response was significant (interaction term between gestational age and pregnancy outcome P value = 0.017). However, a large proportion of the unconditional variation in mean UtA PI comes from case-to-case differences (ρ = 69%).

Table 3.22: Mixed level linear regression to determine relationship between longitudinal uterine artery PI and gestation in prediction of FDIU.

Variable	Coefficient	95% confidence interval	P value
Gestational age (days)	–0.02	–0.004 - –0.001	0.004
Pregnancy outcome FDIU	1.20	0.27-2.14	0.012
Interaction between gestational age and pregnancy outcome	0.006	0.001 – 0.01	0.017
Constant	1.97	1.66 - 2.28	< 0.001

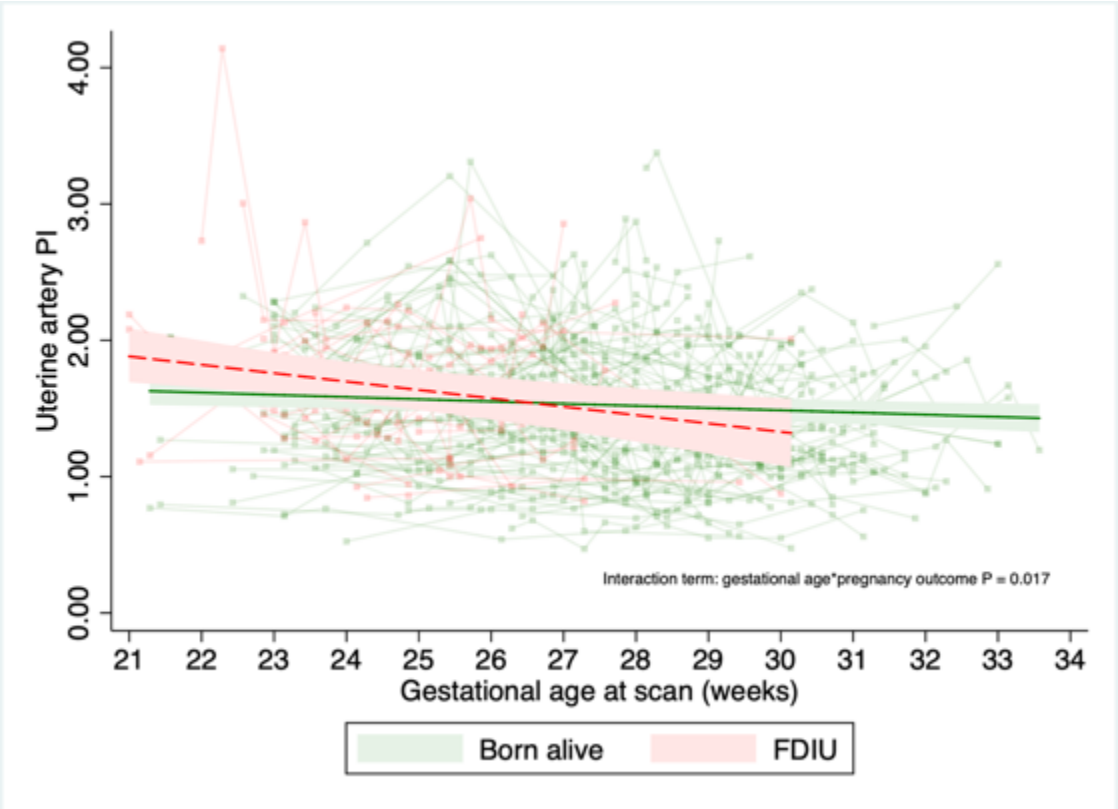


Figure 3.10: Mixed level regression analysis to investigate longitudinal uterine artery change according to live birth/FDIU. Shaded lines represent longitudinal measurements in each individual case. Solid lines represent prediction from regression analysis and 95% confidence interval.

3.3.2.2. Prediction of overall death

A similar relationship was seen when those cases that survived were compared to those cases ending in death (FDIU or NND): there was a non-significant decrease in mean UtA PI across gestation in both outcomes (P = 0.06), and a significant interaction between outcome and gestational age (P = 0.036) (Table 3.23, Figure 3.11).

Table 3.23: Mixed level linear regression to determine relationship between longitudinal uterine artery PI and gestation in prediction of overall death.

Variable	Coefficient	95% confidence interval	P value
Gestational age (days)	–0.002	–0.004 - –0.0006	0.008
Pregnancy outcome Death	0.65	–0.02 - 1.32	0.059
Interaction between gestational age and pregnancy outcome	–0.004	–0.008 - –0.0002	0.037
Constant	2.00	1.66 - 2.34	< 0.001

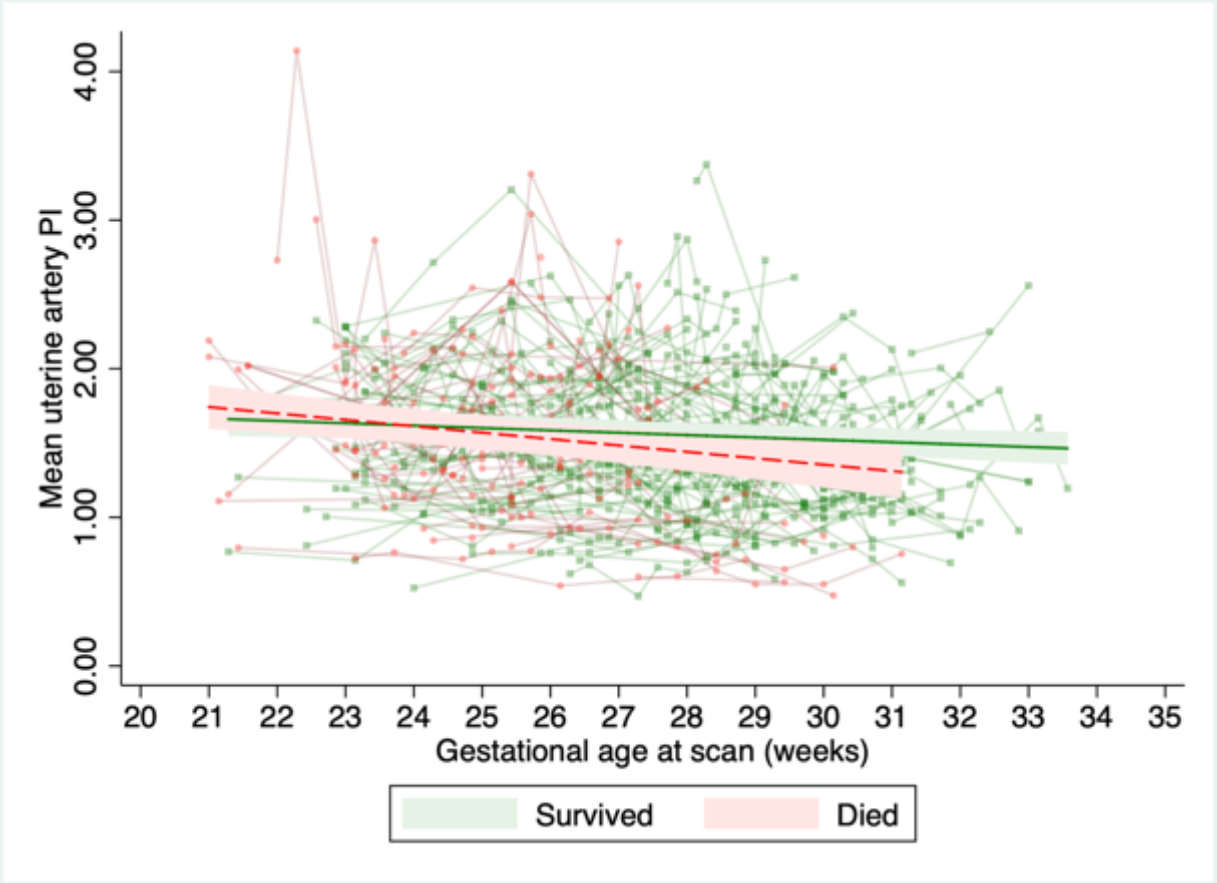


Figure 3.11: Mixed level regression analysis to investigate longitudinal uterine artery change according to survival to discharge/overall death. Shaded lines represent longitudinal measurements in each individual case. Solid lines represent prediction from regression analysis and 95% confidence interval.

3.3.3. *Longitudinal umbilical artery Doppler change*

A total of 593 UA PI measurements were taken from 78 cases of eFGR (outcomes as follows: 72% live birth, survived to discharge/15% FDIU/13% neonatal/infant death). Each case had a median of 3 UA PI measurements taken throughout pregnancy (range 1-19), with a median across all gestations of 1.53 (range 0.84-4.71) (a UA PI cannot be measured when UA EDF is absent or reversed). The results of the regression were not changed using log transformed data, so the data were kept as is.

As previously, the regression was repeated using the two different outcome measures.

3.3.3.1. *Prediction of FDIU*

In those pregnancies ending in a live birth, there was an increase in UA PI as gestation advanced, but this was significantly slower than in those ending in FDIU, as shown by the significant interaction between gestational age and pregnancy outcome (P < 0.001) (Table 3.24, Figure 3.12). As with the UtA Doppler measurements however, a high proportion (68%) of the unconditional variance within the UA PI Doppler measurements is accounted for by case-to-case variability.

Table 3.24: Mixed level linear regression to determine relationship between longitudinal umbilical artery PI and gestation in prediction of FDIU. Gestational age has been included as a cubic term

Variable	Coefficient	95% confidence interval	P value
Gestational age (days)	0.12	–0.21-0.45	0.46
Gestational age*gestational age	–0.01	0.00-0.01	0.52
Gestational age*gestational age*gestational age	$8.76e^{-7}$	$–2.12e^{-6}$-$3.87e^{-6}$	0.57
Pregnancy outcome FDIU	–163.87	–287.16 - –40.59	0.009
Gestational age*pregnancy outcome	2.66	0.61-4.71	0.01
Gestational age*gestational age*pregnancy outcome	–0.01	–0.03 - –0.003	0.01
Gestational age*gestational age*gestational age*pregnancy outcome	$2.54e^{-5}$	$4.83e^{-6}$-$4.61e^{-5}$	0.02
Constant	–7.23	–28.09 - 13.63	0.50

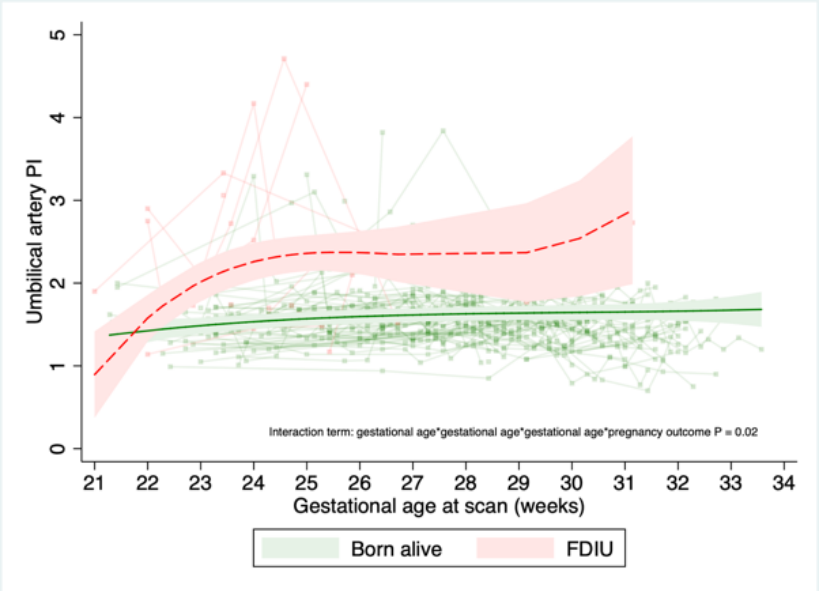


Figure 3.12: Mixed level regression analysis to investigate longitudinal umbilical artery change according to live birth/FDIU. Shaded lines represent longitudinal measurements in each individual case. Solid lines represent prediction from regression analysis and 95% confidence interval.

3.3.3.2. Prediction of overall death

Across all cases there was a significant relationship between gestational age and UA PI, with the UA PI rising as gestation advances (P = 0.001). There was a trend towards a higher UA PI throughout gestation for those cases ending in death compared to those ending in live birth, but this was not found to be significant (P = 0.71), and this relationship did not change with gestation as indicated by the non-significant interaction term between pregnancy outcome and gestational age (P = 0.22) (Table 3.25).

Table 3.25: Mixed level linear regression to determine relationship between longitudinal umbilical artery PI and gestation in prediction of overall death.

Variable	Coefficient	95% confidence interval	P value
Gestational age (days)	0.003	0.001 - 0.005	0.001
Pregnancy outcome Death	–0.18	–1.10 - 0.75	0.71
Interaction between gestational age and pregnancy outcome	0.003	–0.002 - 0.008	0.22
Constant	1.01	0.68 - 1.35	< 0.001

3.3.4. Accuracy of fetal weight estimation

EFW has been highlighted as an important prognostic factor for pregnancy outcome. This relies on both acquisition of accurate images, and the use of a sonographic fetal weight estimation model, which estimates weight based on fetal biometry measurements, therefore it is important to consider how accurate the calculation of EFW is.

65 cases of eFGR where delivery occurred within 48 hours of the last growth scan were included in this sub-analysis. The characteristics of this cohort are summarised in Table 3.26. Of note, overall median EFW calculated using Hadlock BPD-HC-AC-FL was marginally higher than the median birthweight (717g compared to 722g: P = 0.63), highlighting a tendency of the Hadlock BPD-HC-AC-FL model to modestly overestimate fetal weight. Comparing the general characteristics confirms that those cases in which a growth scan occurred within 48 hours of delivery are representative of the overall eFGR cohort, with no significant differences in maternal age (P = 0.79), BMI (P = 0.63), ethnicity (P = 0.40), parity (P = 0.43), gestational age at delivery (P = 0.63) and birthweight (P = 0.63). It should also be noted that pregnancy survival rates of the cases selected for this analysis (76.9%) are higher than the survival rate in the entire eFGR cohort (61.0%), possibly reflecting the nature of antenatal surveillance in this compromised group, with those fetuses too small/early in gestation to survive undergoing less frequent scans until they reach a point of viability.

Table 3.26: Maternal characteristics and pregnancy outcome of overall eFGR cohort and cases selected for inclusion in analysis. Where appropriate, statistical significance between the two groups is quoted.

	Entire eFGR cohort (n = 182)	Cohort selected for final analysis (n = 65)	Significance
Maternal age (years)*	31.1 ± 6.0	31.8 ± 5.97	ns (P = 0.79)
Maternal BMI (kg/m^2)^	27.2 (16.1-46.6)	28.7 (17-41.1)	ns (P = 0.63)
Ethnicity+			
White	88 (49%)	31 (47.7%)	ns (P = 0.40)
Black	23 (13%)	4 (6.15%)	
Asian	31 (17%)	15 (23.1%)	
Other	37 (20%)	15 (23.1%)	
Parity+			
Primiparous	100 (55%)	32 (49.2%)	ns (P = 0.43)
Multiparous	82 (45%)	33 (50.8%)	
Gestational age at delivery (weeks)^	29.4 (22.0-33.0)	29.0 (23.3-32.9)	ns (P = 0.63)
Birthweight (g)^	695 (210-1718)	717 (350-1457)	ns (P = 0.63)
Sex+			
Male	103 (57%)	36 (55.4%)	ns (P = 0.87)
Female	79 (43%)	29 (44.6%)	
Pregnancy outcome+			
Live birth, still alive	111 (61.0%)	50 (76.9%)	P = 0.01
Stillbirth	45 (24.7%)	5 (7.7%)	
Neonatal death	26 (14.3%)	10 (15.4%)	
Study population specific data			
Gestational age at scan (weeks)*	[redacted]	28.6 ± 2.1	[redacted]
Estimated fetal weight at scan (g) ^ (using Hadlock BPD-HC-AC-FL)		722 (357- 1486)	
Amniotic fluid index (cm)* (n = 53)		8.5 ± 4.1	
Fetal presentation at scan+			
Breech		25 (38.5%)	
Cephalic		28 (43.1%)	
Transverse		2 (3.1%)	
Not recorded		10 (15.3%)	

*mean ± SD/t-test quoted for parametric data; ^median (range)/Mann-Whitney quoted for non-parametric data; +counts (percentage of total) Chi-squared quoted for categorical data

Overall, there was a wide variation in the accuracy and precision of the models investigated, with the systematic error having a median of 8.2% (range -87.6-47.5%) and random error a median of 11.6% (range 1.49-23.8%). Figure 3.13 summarises the systematic (box) and random (whisker) errors for each sonographic fetal weight estimation model across the cohort.

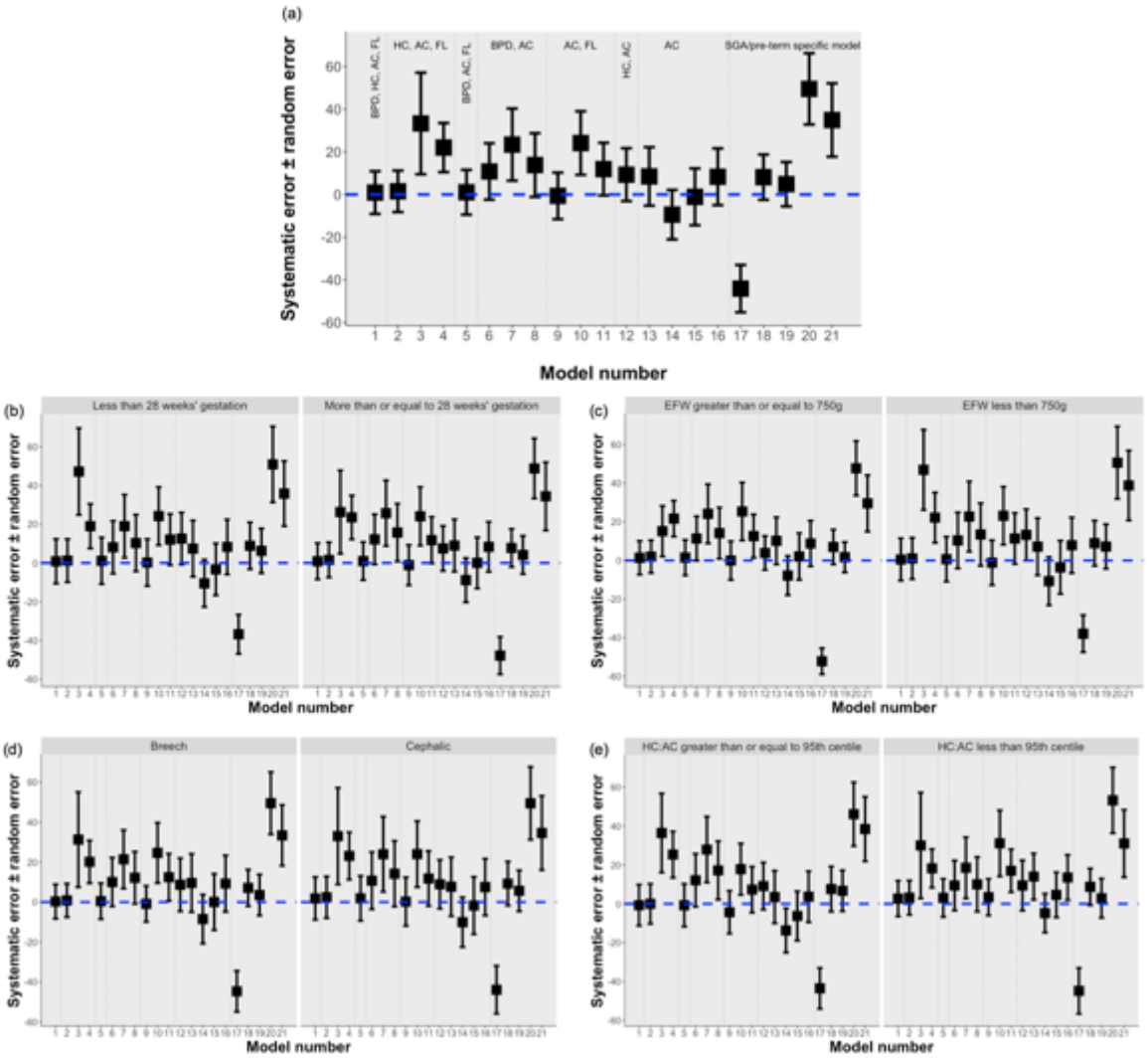


Figure 3.13: Mean systematic error (square) and random error (whiskers) for each sonographic weight estimation model investigated. The blue line represents a systematic error of 0; models plotting above the line show overestimation, models plotting below this line show underestimation of the fetal weight. **(a)** Entire cohort; **(b)** Cohort split by gestational age at scan (pre/post 28 weeks' gestation); **(c)** Cohort split by estimated fetal weight at scan (above/below 750g); **(d)** Cohort split by fetal presentation at scan; **(e)** Cohort split by symmetrical/asymmetrical FGR.

It appears that there is a tendency for overestimation of the EFW, regardless of the fetal biometry parameters included in the model calculation, or the characteristics of the population used to develop the model (SGA or appropriately grown). Splitting the cohort for analysis by gestation (Figure 3.13 (b)), EFW (Figure 3.13 (c)), fetal presentation (Figure 3.13 (d)) or asymmetry (Figure 3.13 (e)) does not change the pattern of over/underestimation, suggesting that none of these factors significantly impact model performance. Systematic error is plotted against random error for the overall cohort, and by gestational age, EFW, fetal presentation and asymmetry (Figure 3.14), but none of these parameters appear to have a significant impact on the accuracy or precision of the models investigated. Figure 3.14 (c) and (d) suggests there is a trend towards improved accuracy in those fetuses with EFW <750g, and those in the breech position, as shown by the split in the groups which is starting to become apparent on the scatter graphs. However, comparing the systematic and random errors, and distance to the origin within the split cohort shows that these factors have no significant influence on model performance.

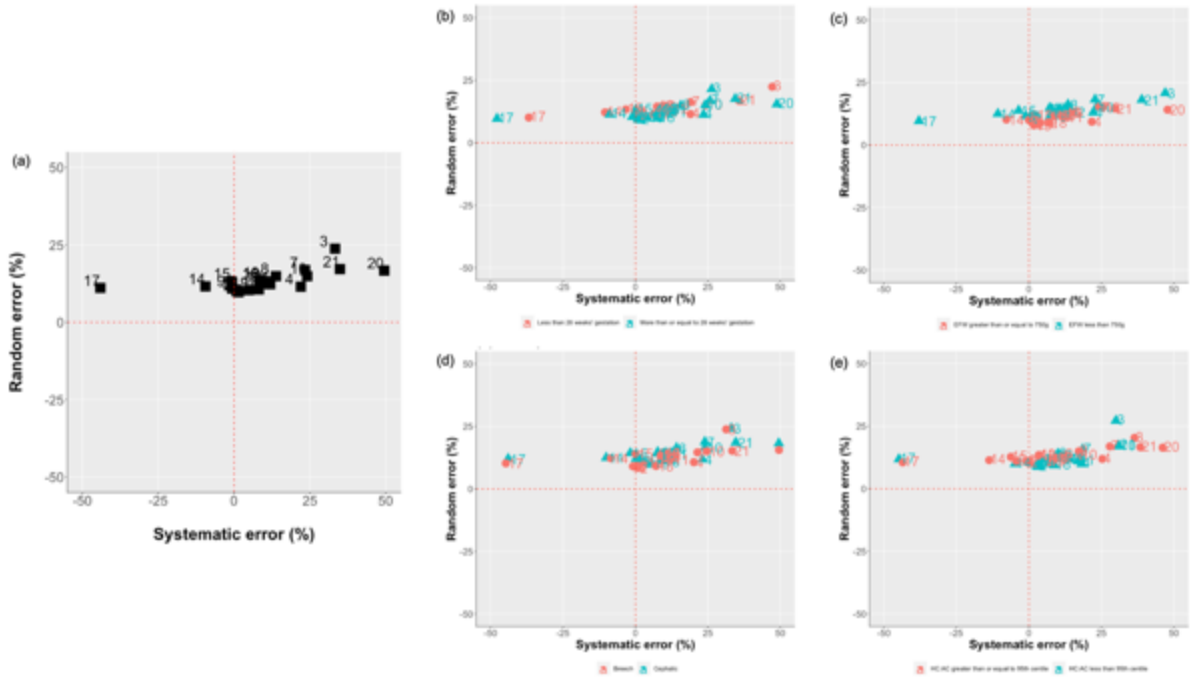


Figure 3.14: Systematic error plotted against random error for models 1-21. Red lines denote systematic error and random error of 0%. Number and colour denote model number as shown in figure legend. **(a)** Entire cohort; **(b)** Cohort split by gestational age at scan (pre/post 28 weeks' gestation); **(c)** Cohort split by estimated fetal weight at scan (above/below 750g); **(d)** Cohort split by fetal presentation at scan; **(e)** Cohort split by symmetrical/asymmetrical FGR.

Table 3.27 summarises the top 3 performing models overall, and according to gestation/EFW/fetal presentation/asymmetry. Model 2 (Hadlock HC-AC-FL) consistently performs the best, regardless of gestational age, fetal presentation or asymmetry. When model performance was assessed according to EFW (less than/greater than or equal to 750g), Model 19 (Schild HC-AC-FL) performed better in those cases where the EFW $\geq$750g; this model was specifically developed for SGA pregnancies, in a cohort with a mean birthweight of 997g, similar to the subgroup investigated here.

Table 3.27: Top three performing models overall, and when the cohort is split by gestational age, EFW and fetal presentation. Number in brackets denotes distance to origin, which is calculated as a composite score to include both systematic and random errors, with a perfect model with a random error of 0% and a systematic error of 0% having a distance to the origin of 0. Mann Whitney used to determine if distance to origin differs within each category in the split cohort. Model 1: Hadlock BPD-HC-AC-FL; model 2: Hadlock HC-AC-FL; model 5: Hadlock BPD-AC-FL; model 9: Hadlock AC-FL; model 19: Schild HC-AC-FL.

	Rank 1	Rank 2	Rank 3	Significance
Overall	Model 2 (9.8)	Model 1 (10.0)	Model 5 (10.6)	
Gestational age < 28 weeks	Model 2 (11.2)	Model 1 (11.5)	Model 5 (12.1)	NS P = 0.62
Gestational age ≥ 28 weeks	Model 2 (9.2)	Model 1 (9.4)	Model 5 (9.9)	
EFW < 750g	Model 2 (10.8)	Model 1 (11.0)	Model 5 (11.6)	NS P = 0.30
EFW ≥ 750g	Model 19 (8.1)	Model 2 (8.7)	Model 1 (8.8)	
Fetal presentation: cephalic	Model 2 (10.7)	Model 1 (10.9)	Model 5 (11.4)	NS P = 0.52
Fetal presentation: breech	Model 2 (8.5)	Model 1 (8.6)	Model 5 (9.0)	
Symmetrical FGR (HC:AC < 95th centile)	Model 2 (9.4)	Model 1 (9.5)	Model 9 (10.0)	NS P = 0.62
Asymmetrical FGR (HC:AC ≥ 95th centile)	Model 2 (10.3)	Model 1 (10.6)	Model 5 (11.0)	

Following identification of the best performing models (Hadlock HC-AC-FL, Hadlock BPD-HC-AC-FL, Hadlock BPD-AC-FL, Hadlock AC-FL, Schild HC-AC-FL), the proportion of cases with an EFW within 5-15% of actual birthweight was calculated. Table 3.28 shows that even with the best performing model (Hadlock HC-AC-FL), only 64.6% of cases have an EFW within 10% of the actual birthweight, and 14% of calculated EFWs are >15% from actual birthweight.

Table 3.28: Proportion of estimates within 5/10/15% of birthweight for top five performing models.

Model	Within 5%	Within 10%	Within 15%	Above 15%
2	41.5	64.6	86.2	13.8
1	41.5	60.0	83.1	16.9
5	36.9	56.9	81.5	18.5
9	36.9	46.1	76.9	23.1
19	36.9	49.2	78.5	21.5

For Model 2 (Hadlock HC-AC-FL), linear regression analysis was used to investigate the continuous relationship between percentage error and gestational age at scan, EFW and AFI (Figure 3.15). No significant relationship was found between percentage error and gestation age (P = 0.381, adjusted R^2 = -0.0035), but there was a weak relationship with AFI (P = 0.019, adjusted R^2 = 0.084). This suggests that at the lowest AFI, Model 2 tends to overestimate fetal weight, but at the highest AFI it tends to underestimate weight.

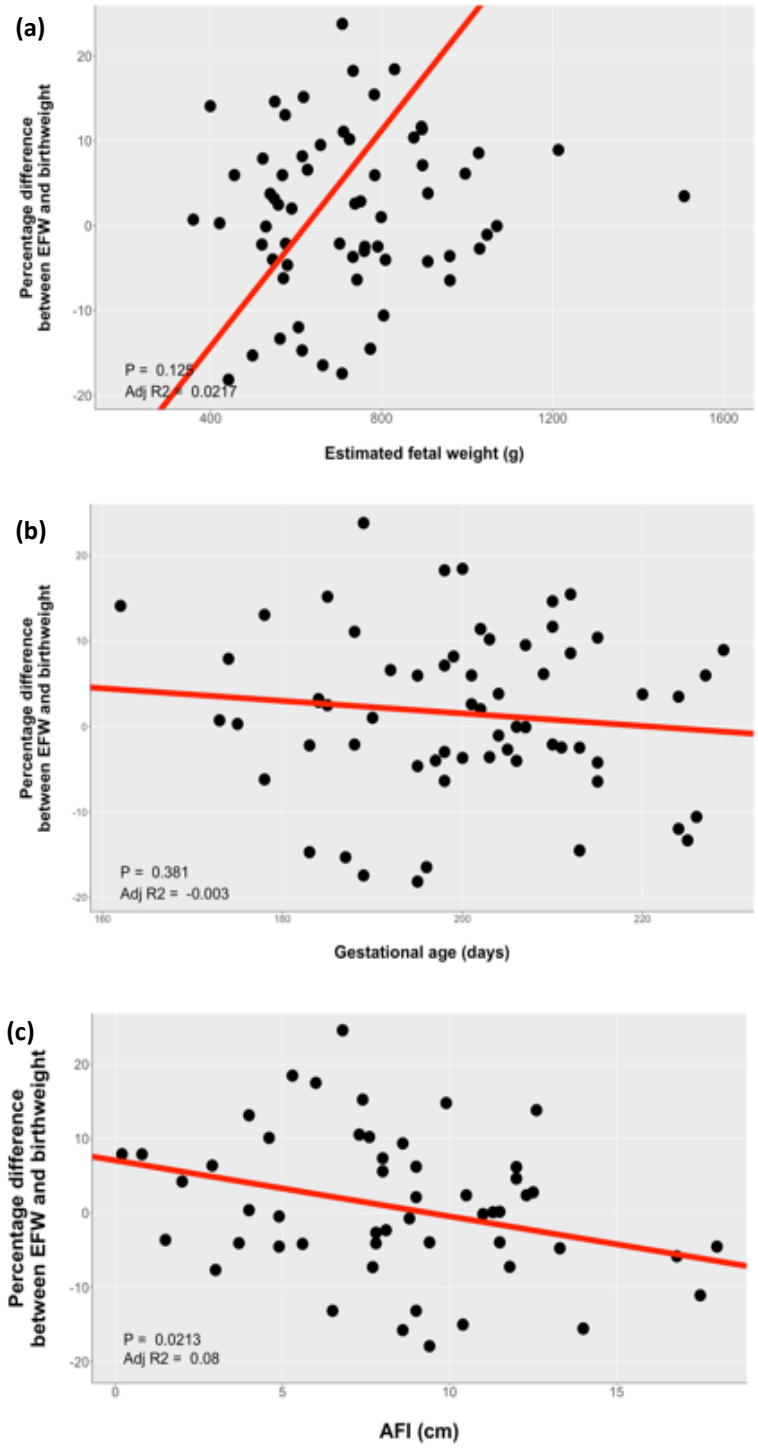


Figure 3.15: Model 2 percentage error against **(a)** gestational age at time of scan for each case (n=65) using Model 2. Regression analysis confirms that there is no relationship between the two (P = 0.38), suggesting that the percentage error does not change with gestation; **(b)** EFW for each case (n=65) using Model 2. Regression analysis confirms that there is no relationship between the two (P = 0.13), suggesting that the degree of error is not related to the actual fetal size in eFGR; **(c)** AFI at time of scan for each case (n=53) using Model 2. Regression analysis confirms an inverse association between the two (P < 0.05), so a low AFI will tend towards overestimation of the fetal weight, and a high AFI will tend towards underestimation of the fetal weight.

3.4. Discussion

3.4.1. Principal findings

The work presented in this chapter aimed to determine factors which are prognostic of pregnancy outcome in cases of eFGR. In order to explore this, it was necessary to first better characterise the eFGR cohort, in order that potential prognostic factors (including factors representing longitudinal change throughout pregnancy) could be identified and quantified in relation to pregnancy outcome. One of the outcomes from this work was a multivariable model that could be used in clinical practice to predict pregnancy outcome in eFGR.

This study has characterised the eFGR cohort, in terms of both maternal characteristics and ultrasound parameters. The analysis has identified factors which are prognostic of pregnancy outcome, gestation at delivery and birthweight, and confirm that a combination of ultrasound parameters can predict FDIU in cases of eFGR. Internal validation and adjustment for optimism suggest that the multivariable model is not perfect (C-statistic of 0.90), but this represents the first step in developing a clinically relevant risk prediction model that can be used to predict pregnancy outcome in patients following a diagnosis of eFGR. Use of longitudinal biometry/Doppler data also suggests a potential prognostic factor that can be continually updated as the pregnancy progresses to reflect any change in risk.

3.4.2. Strengths and weaknesses

This study has two main strengths. Firstly, to our knowledge this is the largest detailed cohort study to determine the prognosis of eFGR to date. Secondly, the data captured include a high level of detail both in terms of the number of ultrasound parameters recorded and longitudinally in terms of the repeated measures. None of the parameters investigated as predictive factors are unique to a research clinic setting and thinking ahead to a model that can be used in clinical practice, it is important that the variables selected as predictors are parameters that can be measured in a typical clinical environment. The collection of longitudinal data has enabled the incorporation of change in variables into prognosis in addition to static factors measured at the time of diagnosis, which represents an additional method of prognostic accuracy that can be updated as the pregnancy progresses. Overfitting was partially addressed through optimism-adjustment.

There are also several limitations to the study. Although this is the largest detailed cohort study to date, having an even larger cohort would increase the number of events (i.e. deaths), which in turn would improve the accuracy of the model and potentially allow incorporation of more variables into the model, which at present is limited by the event rate. Secondly, although permission has been granted to collect data from additional sites, the data analysis presented in this thesis was all collected from one tertiary centre unit (with the exception of the longitudinal fetal biometry data). The model developed using this cohort therefore may not be applicable to a cohort from a different geographical area. This leads to the third limitation, which is that any model needs to be subjected to a process of external validation. Ideally, this would be in a prospectively collected cohort but due to the previously estimated incidence of eFGR of 3 per 1000 cases (Chapter 2), this would likely require a long data collection period from a number of hospital sites. Due to the retrospective

nature of the data collection, the database required extensive cross-checking and data cleaning, and where data points relating to ultrasound measurements were missing there was unfortunately no way of retrospectively obtaining these measurements. A prospective study would also minimise this type of data loss. Finally, potential predictors that have been suggested by other studies include pre-eclampsia status (210) and sFlt:PlGF ratio (52,211). Unfortunately, pre-eclampsia status was not reliably recorded in this retrospective cohort across the whole of the study period. Data relating to indication for delivery suggested that pre-eclampsia was a key factor in 25/182 cases (14%), but these data were only recorded from 2016 onwards. The sFlt:PlGF ratio has only been incorporated into NICE guidance for suspected pre-eclampsia within the last 12 months (53) so the predictive ability of sFlt:PlGF in this cohort could not be assessed. Finally, because of the retrospective nature of this study and the lack of a unified management strategy, the clinical decision-making process cannot be accounted for.

3.4.3. Maternal characteristics

To our knowledge, no previous studies have published data relating to maternal booking characteristics in an eFGR cohort. The only characteristic that was significantly different between pregnancy outcomes was booking diastolic BP (and therefore MAP), which was higher in those cases that ultimately ended in FDIU. Although not significant, a higher proportion of women who went on to have an FDIU were classed as hypertensive (>140/90) at booking. In terms of candidate predictive factors, diastolic BP and MAP were predictive of both pregnancy outcomes (FDIU and overall death), gestation at delivery and birthweight. Chronic hypertension is associated with the development of MVM,(212) which is one of the main placental pathologies implicated in eFGR (213) , and therefore could predispose an individual to a more severe phenotype of eFGR. Women presenting with chronic hypertension are more likely to develop pre-eclampsia during their pregnancy,(214) also potentially leading to a more aggressive phenotype of eFGR. In the presence of severe pre-eclampsia, delivery may be necessary to treat the maternal disease regardless of the fetal weight or gestation, and in some cases of eFGR induction of labour will be recommended despite the inevitable outcome being FDIU, for example, where the EFW is predicted to be less than 400g.

3.4.4. Ultrasound characteristics

The ultrasound characteristics identified as candidate prognostic factors or as predictive of gestation at delivery or birthweight are all biologically plausible as they are all indirect measures of placental function. However, quantification of these relationships is helpful in moving towards creating a predictive model for clinical use.

All of the fetal biometry measurements and EFW were significant predictors of both FDIU and overall death, even when corrected for the gestation at which they were measured. Growth, and eventual birthweight, is one of the biggest predictors of survival, as discussed in Chapter 2, therefore it would be expected for a relationship between fetal size and pregnancy outcome to exist, even within such an extreme cohort of FGR. A factor that is potentially surprising is the magnitude of the change associated with each biometry measurement. A 5mm change in BPD or FL results in a large reduction in FDIU/overall death risk, although this is likely related to the size of

the structure being measured in the first instance, with the BPD and FL measurements significantly smaller than the HC or the AC.

It could be argued that using EFW as a candidate prognostic factor in a multivariable model is inappropriate, as EFW itself is not a directly measured variable, but is the product of a linear regression equation including the fetal biometry measurements. In this situation, however, it seems appropriate as it represents a composite of all of the fetal biometry measurements which have all been shown to be significantly predictive of outcome. In a clinical sense, it is a value that can be calculated even if one or more of the fetal biometry measurements are missing or unable to be accurately measured at the time of scan. In terms of centile, interestingly using the non-customised centile appears to reach a higher level of statistical significance, with a one centile change resulting in a greater risk difference when compared to customised centiles. Again, this is likely to reflect the extreme nature of this cohort, whereby adding in maternal characteristics to calculate centile is unlikely to result in any meaningful change in the centile value, as they are all skewed towards the very lower end.

Amniotic fluid is a marker of fetal wellbeing, and is associated with FGR (215). It is likely reflective of decreased fetal urine production, secondary to placental insufficiency (216), but another causative mechanism that has been proposed is an alteration in the solute concentration of amniotic fluid which results in excess intramembranous reabsorption of the amniotic fluid (217). Although no measures of amniotic fluid were selected for the final predictive model, the observed association emphasises the fact that it is an important part of the ultrasound assessment of fetal wellbeing, and amniotic fluid should be objectively quantified in cases of eFGR even if subjectively the volume appears normal.

Umbilical artery flow represents resistance in the fetoplacental vascular bed (218) and is a well-established measure of fetal wellbeing, as discussed in Section 1.5.2.3.2. It is therefore expected that higher resistance in the UA at the time of diagnosis is associated with a higher chance of adverse outcome. Only 46% of cases had UA EDF present at the time of diagnosis, which means firstly that this association can only be confirmed in a subset of the whole cohort, and secondly that UA EDF status (e.g. present/absent/reversed) rather than a numerical measure of the resistance is a more inclusive method of predicting outcome. By only considering those cases with a measurable UA PI where UA EDF is present, the odds ratios are biased to those cases at the less extreme end of the spectrum and are not representative of the whole eFGR cohort. Nevertheless, the odds ratios for the predictive value of UA EDF status reflect current thinking that UA Doppler generally deteriorates in a predictable manner from present to absent through to reversed (121). Given the small number of cases with reversed EDF at presentation, the confidence intervals for both prediction methods (FDIU/overall death) are wide, and although statistically significant, the coefficients are potentially inaccurate. For this reason, classifying UA EDF as normal (present) or abnormal (absent/reversed) was chosen as the most appropriate UA measure to be tested in the multivariable prediction model. Given the association between DV a-wave and mortality (138), this may have been expected to be a potential candidate predictor. However, only 9 cases in the cohort

had an abnormal a-wave at the time of diagnosis. The low proportion of cases with an abnormal a-wave at presentation likely reflects the fact that the majority of these cases were pre-viable in terms of gestation and / or EFW and therefore not at a point where delivery could be offered.

In this cohort, a high proportion of cases were expected to have abnormal UtA resistance because of the preponderance of histopathological lesions of MVM in eFGR (29). However, mean UtA resistance only showed prognostic value in the prediction of FDIU, not overall death. If the mean values of UtA PI are considered by pregnancy outcome, the values are lowest in those pregnancies ending in neonatal/infant death (1.4 $\pm$ 0.5), and highest in those ending in FDIU (1.7 $\pm$ 0.6). This finding explains why mean UtA resistance is only prognostic of FDIU, rather than overall death. Physiologically, this difference may represent differences in the distribution of pathological causes of eFGR, with FDIU more likely to be associated with MVM, and those pregnancies that are delivered alive, but unfortunately die following delivery caused by other pathological processes or combination of processes, or possibly undiagnosed genetic or structural problems that do not manifest until the postnatal period. Although the PI approaches statistical significance, it is the UtA RI that is shown to be a better indicator of prognosis. This is reflective of the fact that RI is more mathematically stable than PI and is calculated as a ratio of the difference in peak systolic and diastolic variables, whereas PI also takes into consideration the average velocity. RI will always be measured between 0 and 1, whereas PI can increase exponentially.

3.4.4.1. Prediction of birthweight and gestation at delivery

Similar relationships were found when the ability of maternal characteristics and ultrasound parameters to predict gestation at delivery and birthweight were tested. The findings in Chapter 2 confirmed the importance of birthweight in predicting outcome. Therefore, if we are able to reliably predict eventual birthweight then this will give clinicians a useful prognostic indicator of survival. It is unlikely, however, that a scan at the time of diagnosis will give sufficient information to be able to accurately predict birthweight when the pregnancy may continue for another 8-10 weeks in some cases. This highlights why incorporation of longitudinal data into any model is an important factor to reflect changes in placental function that occur throughout gestation and cannot be modelled at the time of diagnosis.

3.4.4.2. Multivariable analysis to predict pregnancy outcome

The prognostic factor study has allowed development and internal validation of a multivariable model to predict pregnancy outcome following diagnosis of eFGR. The parameters included within the models are all routine clinical measurements, which could be undertaken in any hospital obstetric ultrasound department. Such a model could be used at the time of diagnosis of eFGR to predict the likelihood of livebirth/FDIU; although this does not give a clinician an indication of when exactly to deliver, it does provide significant information about the prospects of the pregnancy to parents and managing clinicians. The model proposed to predict FDIU had a negative predictive value of 96% meaning that clinicians could reassure affected parents that if the model predicts the pregnancy will result in a live birth then the chance of an FDIU happening is small (4%).

Comparison of the AUC and apparent performance statistics suggests that the model to predict FDIU performed better than the model to predict overall death. Looking at the summary of ultrasound characteristics at the time of diagnosis and the univariable analysis results (Section 3.3.2) suggests that there are differences between the FDIU cohort and the neonatal/infant death cohort. This indicates that although both end in death, it could be inappropriate to group these two populations together for prediction purposes. For example, the birthweight in those cases ending in FDIU compared to those cases ending in neonatal/infant death was significantly different (390g compared to 595g; $P < 0.001$, Kruskall-Wallis), as was the gestation at delivery (26.9 weeks compared to 28.7 weeks; $P < 0.001$, Kruskall-Wallis). This also reflects selection bias from the clinician though as some FDIUs will occur in cases where the birthweight is deemed too small for any intervention to improve the outcome, whereas the majority of neonatal deaths will have occurred in cases which were actively managed as they reached a point where survival was felt to be a possibility. If grouped together for prediction purposes, the predictive power of these differences would be lost. It therefore seems appropriate to take forward only the predictive model for FDIU/live birth for further development. An ideal model to differentiate between pregnancies ending in live birth, FDIU and NND would take the form of a multinomial logistic regression model, able to discriminate between the three outcomes of interest, but there is insufficient statistical power in the dataset here to facilitate that at present.

Reassuringly, a model to predict short-term neonatal outcomes, based on the STRIDER population also found EFW to be predictive of livebirth and neonatal morbidity (210). This model also highlighted the predictive value of pre-eclampsia status and the sFlt:PlGF ratio in prediction of outcome in eFGR, which as previously mentioned, this retrospective study was not able to assess. UA EDF status was not found to be predictive of pregnancy outcome, however the inclusion criteria for STRIDER stipulated that UA EDF was absent or reversed at the time of randomisation (15). As this analysis was part of the STRIDER study, data was collected prospectively and although based on a smaller cohort of eFGR patients (n = 135) from multiple UK sites, had similar rates of pregnancy outcomes and was collected over a smaller time period (November 2014-July 2016), therefore will be less influenced by changes in management practice over time (15,210). Similar to the cohort analysed in this chapter, eFGR management was undertaken by the local clinical team and the STRIDER protocol did not dictate a unified management strategy, therefore outcomes may have been biased by clinician input. The cohort recruited to the STRIDER study does represent a potential prospectively collected dataset on which the predictive model developed in this study could be externally validated.

3.4.4.3 Longitudinal changes

The analysis performed looking at longitudinal data to predict pregnancy outcome provides new methods of relating growth and Doppler changes to pregnancy outcome. In terms of fetal growth, this study has shown not only that size is consistently smaller throughout pregnancy, but also that the fetal growth rate was slower in those pregnancies that end in FDIU or overall death. By identifying those gestational age periods where the growth rate was associated with pregnancy outcome (24-25^{+6} weeks' and 26-27^{+6} weeks' gestation) and assessing the rate of growth in those

periods, it has been possible to relate a quantifiable fetal weight gain to a decreased risk of adverse outcome. This information can be used to reassure both parents and clinicians that in those cases where there is at least a weekly weight gain of 50g between 24 and 27^{+6} weeks' there is a reduction in the likelihood of FDIU or neonatal/infant death by approximately 50%. This is potentially a more informative measure of fetal wellbeing in eFGR than plotting fetal growth on a standard fetal growth chart as the majority of infants in this cohort will plot well below the 5^{th} centile line on either a customised or population growth chart, and they are not likely to grow at a rate that follows the expected curve of the line. Therefore, other than for demonstrating that growth between two scans has not been static, arguably there is little to be gained from using a standard fetal growth chart in eFGR. Previous work has suggested a mean fetal growth rate from 24 weeks until delivery of 176.5g per week, or 169.4g per week in a high risk population (25.2g and 24.2g per day respectively) (99). A study looking specifically at growth in the third trimester produced an average growth rate value of 24.2g per day, which was reduced to 21.9g per day in pregnancies with an abnormal outcome (101). Given the extreme nature of the eFGR cohort, however, these standards should not be applied to growth in eFGR where a much lower amount of fetal growth appeared protective. Further work needs to be undertaken to determine what the minimum required growth in eFGR is to be confident of a live birth, but from this study a growth rate of at least 50g per week (between 24 and 27^{x} weeks' gestation) can be taken to indicate a 50% reduction in the risk of FDIU or death.

The longitudinal Doppler analysis provides useful and interesting insight into changes throughout an eFGR pregnancy. Due to limitations with the data discussed previously, these data in their current form may not be sufficient to support implementation of such a model into clinical practice at present.

The analysis performed here suggests that for pregnancies that ended in live birth or overall survival, the mean UtA PI remained relatively constant throughout gestation, showing a small but statistically significant decrease. This contrasted with those eFGR pregnancies ending in FDIU or overall death, which showed a more pronounced decrease across gestation, and started higher but fell more quickly to end up lower at later gestations. The data are lacking in generalisability at the later gestations however, as 50% of the FDIU cases are delivered by 27 weeks' and the neonatal/infant death cases by 28.7 weeks' gestation. This may in part reflect selection bias as discussed previously. This means that the number of data points beyond these gestations in the FDIU/overall death outcome groups was much smaller than the live birth outcome groups which was reflected in the wider confidence intervals at the later gestations. With fewer data points at the later gestations, the regression line is likely to have been influenced by outliers and so may not be representative of the true pattern.

The same problems as described with the UtA Doppler analysis complicate the longitudinal analysis of UA PI. In addition, analysis of UA PI does not take into consideration UA EDF status as UA EDF must be present to calculate a PI value. Thus, the pattern of changes in UA PI only represents changes in UA resistance at the less severe end of the disease spectrum, and only

39% of eFGR cases had EDF present at the time of diagnosis. Therefore, longitudinal measurement of PI is biased towards those cases likely to have a better outcome, which was evident as the pregnancy outcomes of cases included in the UA PI longitudinal analysis were different to the total cohort outcomes. However, assigning a binary figure to EDF status and using this in a mixed level regression to assess EDF change throughout pregnancy did not show a relationship with gestation, nor a difference depending on pregnancy outcome. This is likely due to the frequency with which UA EDF status can change; within our dataset it was not uncommon for UA EDF to deteriorate to absent, only for it to be present at the following visit.

3.4.4.4. Accuracy of fetal weight estimation

This study has shown that despite the fact it was developed using a relatively small (n = 276) but predominantly Caucasian population of mixed gestational age fetuses, with a range of fetal weights, the Hadlock HC-AC-FL sonographic weight estimation is the most appropriate to use in this eFGR cohort. This model remained the most appropriate choice regardless of gestational age, EFW, fetal presentation or asymmetry, or liquor volume status.

The slight increase in performance in Hadlock HC-AC-FL over Hadlock BPD-HC-AC-FL (currently standard practice in the translational research clinics in this unit) confirms that addition of BPD does not confer increased accuracy or precision of EFW calculation. However, the margin of improvement gained from excluding BPD measurements is minimal, so it would be unreasonable to conclude the inclusion of BPD is detrimental to EFW calculations. There does not appear to be a significant benefit in measuring BPD, however, and the time saved by not measuring BPD could have implications in terms of scan time and efficiency.

This study highlights that there is a need for an improved model of fetal weight estimation to reduce the number of cases where there is an error of more than 10% between estimated and actual birthweight. Such a model could either use routinely measured fetal biometry parameters and be developed within a cohort of eFGR pregnancies, or it could incorporate alternative fetal biometry parameters, for example three-dimensional thigh volume measurements (219).

3.4.5. Implications for clinical practice

Providing a prognostic estimate on an individual level permits better discussion with affected families about expected pregnancy outcomes. The risk prediction models developed here have been developed in the context of current clinical care, and will have been influenced by decisions surrounding delivery, which may differ between clinicians and may have changed over time. Due to the retrospective nature of this study, this cannot be controlled for in this cohort. However, these models are not designed to act as tools to influence timing of delivery, they are designed to give information on likely outcomes, and the work presented here shows that we can start to give some indication of babies which we think are likely to die and those which are not. Not only does this give affected parents more information can be offered at present, but it will allow clinicians to prioritise scan resources if a situation arises where there is insufficient scan capacity to accommodate all cases. It is not be anticipated that this would be a tool to determine if termination of pregnancy is an appropriate option. Although termination is offered in selected cases in eFGR (17), this tool

does not predict with certainty the outcome of FDIU and opting for conservative management allows the pregnancy to gain gestation and reach a point whereby a live delivery may be possible. This study has also highlighted that in terms of ultrasound measurements, Hadlock HC-AC-FL provides the most accurate estimation of fetal weight. In the absence of an improved formula, this should be the sonographic weight estimation model of choice.

3.4.6. Future work

At the start of the study, the decision was made to collect all available data relating to eFGR through the translational research clinics at St Mary's Hospital. As discussed, this has provided an estimation of the outcome rates associated with eFGR and identified potential prognostic factors, and this data can be used to guide further model development. To improve on this and investigate the inclusion of additional predictor variables, the development cohort size needs to be sufficiently large to minimise overfitting and generate precise variable coefficients (220). Based on the overall proportion of eFGR pregnancies that will end in FDIU (0.25), the potential number of candidate predictors (10; based on five identified in this study and five further potential factors), R^2_{CS} 0.3 (based on this model's adjusted R^2 0.36), and a shrinkage factor of 0.9, the minimum sample size required for new model development would be 289, with 73 FDIU events and an events per prediction parameter of 7.23 (221). Given that the study discussed in this chapter collected data from 182 cases over a 10-year period and the relative infrequency of eFGR (3 per 1000 births), such a study will require collaboration between multiple large maternity units. External validation of a prognostic model requires a slightly different approach. It has been suggested that an inadequate sample size leads to inappropriate decisions to discard a prognostic model, therefore it is recommended that the external validation cohort includes a minimum of 100 events (FDIUs in this instance) (222). Meta-analysis techniques can be used to combine data from multiple studies to externally validate a model and account for heterogeneity between studies (223), therefore using both the TRUFFLE and STRIDER datasets represents a potential method of externally validating the model proposed here, if an existing dataset were to be used.

Following validation of a predictive model, its clinical impact should be assessed. This would require a prospective cohort study, in which the model is used at the point of diagnosis in new cases of eFGR and the predicted and actual outcome of the pregnancy recorded. The predicted result would need to be concealed from the clinical team to prevent this from influencing their management decisions and impacting on the pregnancy outcome. Such a study could take the form of a stepped wedge cluster randomised trial which have been used successfully in previous obstetric intervention studies (49,224), in which participating maternity units (clusters) are randomised to blocks. Due to the low incidence of eFGR, participation from a large number of maternity units would reduce the study period and reduce the risk of changes in management practices over time as a confounding factor. At the initiation of the study, all units would use the model, but the predicted outcome would remain concealed. Successive blocks would then transition to having the predicted outcome revealed and the pregnancy subsequently managed as per a standard algorithm depending on the prediction result. A consortium obstetric / neonatal opinion would be sought as to the best management strategy for both outcomes from the

algorithm. Outcome measures of interest would include the FDIU rate, neonatal mortality and morbidity, interval from diagnosis to delivery, gestational age at delivery, birthweight, estimated costs of antenatal management and measures of patient satisfaction. In order to have a positive clinical impact, it would be hoped firstly that revealing the prediction was acceptable and beneficial to both parents and clinicians. Secondly, any negative clinical impact would want to be identified – for example if it led to a rise in neonatal mortality or morbidity secondary to a reduction in the interval from diagnosis to delivery due to increased concern about the likelihood of an FDIU. Finally, the effect the revealed result had on scan resources would need to be assessed to ensure it was resulting in more appropriate allocation of scan resources.

The work presented here represents two separate models: a model that can be used at the time of diagnosis and the use of longitudinal data to look at prognosis (either through fetal growth trajectory or umbilical artery Doppler change). The next step would be to combine these parameters into one model that can be used throughout the duration of the pregnancy and would be continually updated to provide a "real-time" estimation of survival chances or risk of death.

Finally, this study considered short-term neonatal outcomes only. There is a need for further research surrounding long-term neonatal outcomes in eFGR. A prospective study such as that outlined above, could also involve an extended postnatal follow up study of eFGR infants that survive to two years. Such a study would reveal if any antenatal characteristics or ultrasound parameters are predictive of specific neonatal morbidities.

3.4.7. Conclusions

This study has provided previously lacking information regarding characterisation of the eFGR cohort. From this, we have been able to identify potential individual prognostic factors at the time of eFGR diagnosis that show promise when used in combination to predict the likelihood of FDIU. This model works on a similar concept to a previously published model based on the STRIDER cohort (210), although it uses different predictor variables. The idea of the model developed is not to dictate the antenatal surveillance or time of delivery, but to guide parents, obstetricians and neonatologists about the chances of a positive outcome from the pregnancy. In addition, we have shown how the huge amount of longitudinal data that can be gathered over the course of a pregnancy relating to both fetal growth and fetal/maternal Dopplers can be used to modify risk predictions as pregnancy progresses.

CHAPTER 4: EXPLORATION OF THE EFFECTS OF SINGLE CENTRE eFGR MANAGEMENT EVOLUTION 2009-2019

4.1. Introduction

Over the period of data collection for the datasets used in Chapters 2 and 3, there have been a number of influential studies published within the field of eFGR as discussed in Section 1.4 (15,76). It is inevitable that eFGR management will have changed over time to reflect the findings from these studies, and the data collected for the previous chapters provides the ideal platform from which to investigate these changes at the level of an individual maternity unit. Ideally, as practice evolves to accommodate new research findings, pregnancy and neonatal outcomes will improve.

As with all clinical practice, there are economic implications of clinical decision making and developments in practice. Therefore, evaluating changes in clinical practice can provide information as to the allocation of finite resources. In the case of eFGR, care does not stop at the point of delivery, but responsibility is transferred to the neonatal team. eFGR infants are born at such extremes of gestation and birthweight that a prolonged neonatal stay is inevitable (which has significant resource implications), yet there has been no research assessing the impact of this specifically in the context of eFGR. A French study has suggested that care for a SGA pregnancy costs €2783 more than a normal healthy pregnancy, due to increased use of antenatal resources and longer hospital stays of both mother and baby, and that although SGA pregnancies contributed to 10.9% of births, they accounted for 23% of total costs (225). By investigating neonatal length of stay, more reliable information can be provided to parents regarding the postnatal period, and the wider impact on the NHS in terms of neonatal care can start to be explored. Although eFGR is a problem that starts in the antenatal period, more research is required to assess the impact of this disease in the postnatal period and beyond.

4.2. Methods

Data for this chapter are taken from the databases created for both Chapter 2 (St Mary's Hospital singleton non-anomalous deliveries between 2012-2017), and Chapter 3 (detailed retrospective eFGR cohort from St Mary's Hospital).

4.2.1. Change in practice over study period

To investigate how eFGR management has changed over time, the detailed retrospective cohort data detailed in Chapter 3 were used. The following factors were analysed:

a) Number of cases
b) Number of scans per patient from diagnosis to delivery
c) Diagnosis to delivery interval
d) Time from first episode of absent EDF to delivery
e) Change in pregnancy outcomes.

4.2.1.1. Number of cases

The number of new diagnoses of eFGR for each year from 2009-2018 was identified and the change modelled using a Poisson distribution, to determine if there was a significant difference over the time period. Due to small numbers of cases in the early years of data collection, years were amalgamated as follows: Group 1: 2012-2014; Group 2: 2015-2016; Group 3: 2017-2018.

4.2.1.2. Number of scans per patient

The number of scans that each eFGR case underwent from diagnosis to delivery was calculated. This was further split into growth scans (where fetal biometry was measured), and Doppler only scans (where only maternal/fetal Doppler measurements were performed). Years were amalgamated as described in the previous section. As these data represent count data, Poisson regression was used to model this relationship. For each year, the average number of growth / Doppler / total scans performed per eFGR case was calculated, and this value used in a Poisson regression as the dependent variable, with year included as an independent variable. This regression was adjusted for the interval between the first scan and delivery by including this variable as a covariate. Results are displayed graphically, and as incidence rate ratios (95% CI). Analysis was initially performed by year, then years were amalgamated into year-groups of 2012-2014, 2015-2016 and 2017-2018 to increase event counts and prevent overfitting. Cases prior to 2012 were excluded from the analysis as the numbers in this time frame were small (n = 16).

4.2.1.3. Diagnosis to delivery interval

Linear regression was used to determine how the length of time between “diagnosis” (i.e. first scan with findings consistent with eFGR) and delivery has changed over time. First scan to delivery interval was used as the dependent variable and year/year group as a categorical independent variable. The first scan-delivery regression also included the gestational age at first scan, to account for the variation in gestation at presentation. The regression was repeated excluding those cases that were delivered for maternal reasons to determine if this altered the findings. Results are displayed as OR with 95% confidence intervals. Analysis was performed by individual year and pooled years as described above.

4.2.1.4. Time from first episode of absent EDF to delivery

Linear regression was used to determine how the interval between the first detected episode of absent EDF on scan and delivery has changed over the study period. First episode of absent EDF to delivery interval was used as the dependent variable and year/year group as a categorical independent variable. The gestational age at which absent EDF was first detected was included as a covariate. In many cases, progression from EDF present to absent EDF is not predictable, and although absent EDF may be diagnosed on a scan, EDF may subsequently be present on a later scan. For this reason, the first episode of absent EDF was used to calculate the absent EDF to delivery interval.

4.2.1.5. Change in outcomes

The change in outcome rates (FDIU / neonatal or infant death / overall death) was modelled using a binomial distribution. Logistic regression was performed with pregnancy outcome as the dependent variable and year/year group as an independent variable. Results are displayed as OR with 95% confidence intervals. Analysis was performed by individual year and pooled years as described above.

4.2.2. Neonatal length of stay

Data regarding the neonatal LoS (including both time to discharge for those infants that survived to discharge, and time to death for those infants that died prior to discharge) were also collected as part of the local St Mary's Hospital data set (Chapter 2) using BadgerNet Neonatal Electronic Patient Record Version 2.9.1.0 (Clevermed, Edinburgh, UK). Through this system, the total LoS in a neonatal unit was available, even if the patient was discharged from St Mary's Hospital to another unit. Cases were excluded from this section of the analysis if the infant was still an inpatient at the time of data collection, or if either the discharge destination or the LoS were not available through BadgerNet (which was the case if the infant was discharged to a unit that does not use the BadgerNet system).

Maternal demographic and pregnancy outcome data were produced for this sub-cohort and compared with the overall cohort to determine if there were any significant differences that could account for any bias. The distribution of the LoS variable was checked for normality using the Shapiro-Wilk test. The median (range) was calculated for the whole cohort and then by eFGR status to determine how eFGR affected the neonatal LoS. Gestational age at delivery was split into groups (<24/40, 24-25^{+6}/40, 26-27^{+6}/40, 28-29^{+6}/40, $\geq$30/40) and median LoS calculated by gestational age and eFGR status. This was repeated for birthweight which was grouped between 500 to over 2000g in 250g increments. Time to death was also compared between eFGR and non-eFGR infants, but due to the smaller numbers involved, splitting the cohort for comparison by gestation and birthweight was not feasible.

To investigate the continuous relationship between LoS, and gestation at delivery and birthweight, univariable analysis using a generalised linear model assuming a Gaussian family with an identity link was performed. As described previously, predictors were initially included as a linear term, before investigating whether high order polynomials and inclusion of eFGR status as an interaction term improved the model fit. Reported results are presented as coefficients (95% CI), and graphically.

Finally, linear regression was used to determine how the neonatal length of stay has changed over time. LoS was used as the dependent variable and year/year group as a categorical independent variable. The regression was repeated excluding those cases where the LoS was more than 2 standard deviations away from the mean to determine if the results were influenced by outliers. Results are displayed as coefficients with 95% confidence intervals, and graphically.

4.3. Results

4.3.1. Change in practice over study period

4.3.1.1. Number of cases

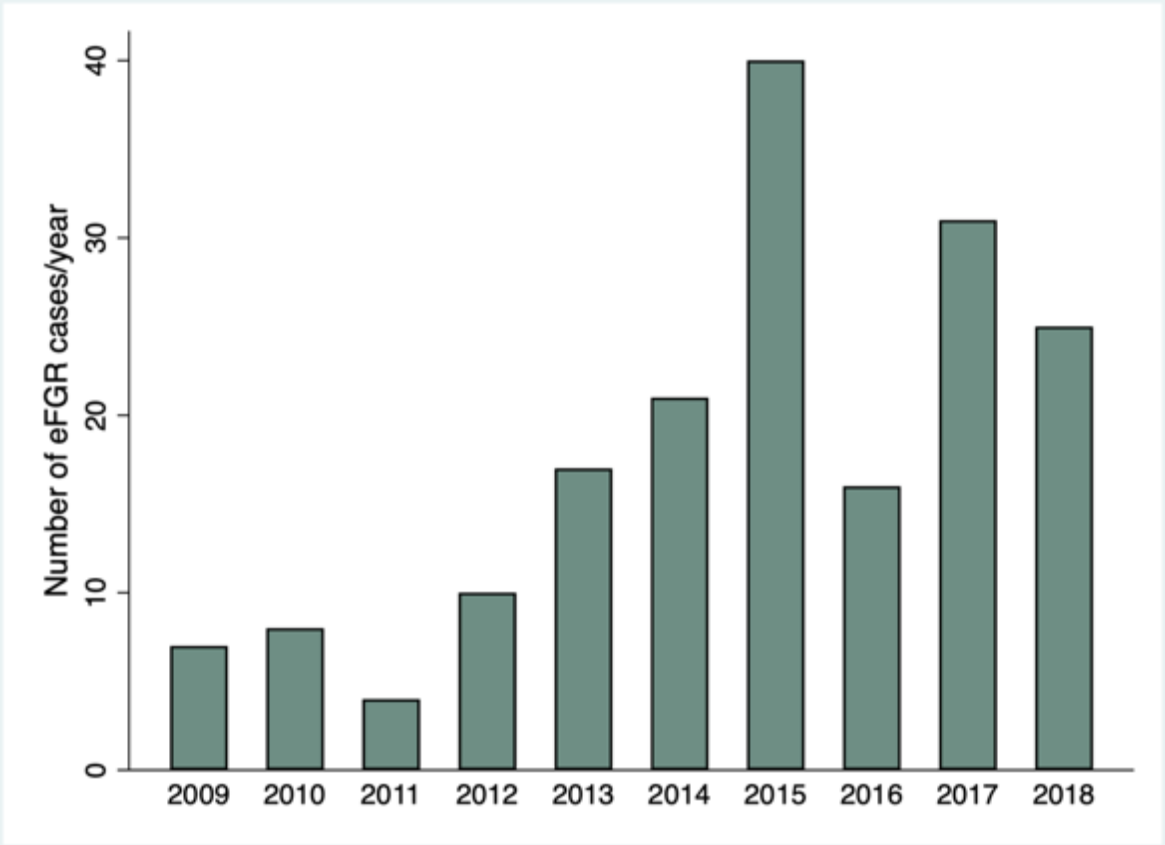


Figure 4.1: Number of eFGR cases managed through the translational research clinics at St Mary's Hospital, Manchester between 2009-2018.

The number of eFGR cases reviewed in the translational research clinics at St Mary's Hospital has risen over the study period, to reach a peak of 40 per year in 2015, which coincided with recruitment for the STRIDER trial. Since then, it has slowed slightly (Figure 4.1).

To smooth out the change in case numbers for the Poisson analysis, years were considered in groups as detailed in the methods (Section 4.2.1.). Table 4.1 summarises how the number of cases has changed over the study period. Cases from years 2009-2011 were excluded from the analysis due to low numbers. There was a significant rise in case numbers from 2012-2014 to 2015-2016 (Incidence Rate Ratio (IRR) 1.99 (1.84-2.17); P < 0.001), then a significant decrease from 2015-2016 to 2017-2018 (IRR 0.86 (0.80-0.92; P < 0.001).

Table 4.1: Change in eFGR case numbers over study period as determined by Poisson modelling. 2009-2011 were excluded from the analysis due to low case numbers. Group 1: 2012-2014; Group 2: 2015-2016; Group 3: 2017-2018.

Time period comparison	IRR (95% CI)	P value
Group 1 → Group 2	1.99 (1.84-2.17)	< 0.001
Group 1 → Group 3	1.71 (1.57-1.87)	< 0.001
Group 2 → Group 3	0.86 (0.80-0.92)	< 0.001

4.3.1.2. Number of scans per patient

Table 4.2 and Figure 4.2 summarise the total number of scans per patient, and the number of growth scans and Doppler only scans per patient. This has increased steadily from 2012 to reach a peak in 2015 of 9.6 scans per patient and has then remained relatively constant. When this is broken down by scan type, the number of growth scans per patient increased to 4.8 in 2016, then has remained constant at 4 per patient, while the number of Doppler scans has increased to a maximum of 7.6 per patient in 2018.

Table 4.2: Yearly summary of total scans / growth scans / Doppler scans per patient in eFGR cohort. Data from 2009-2011 are included but shaded grey as this was not included in the Poisson analysis due to the small number of cases.

Year	Mean total number of scans/patient	Mean number of growth scans/patient	Mean number of Doppler scans/patient
2009	5.6	3.6	2.0
2010	6.1	4.3	1.9
2011	4.5	2.0	3.3
2012	5.0	2.7	2.6
2013	5.4	2.9	3.2
2014	7.9	3.5	5.7
2015	9.6	4.2	6.2
2016	9.4	4.8	6.1
2017	8.4	3.6	6.0
2018	10.6	3.9	7.6

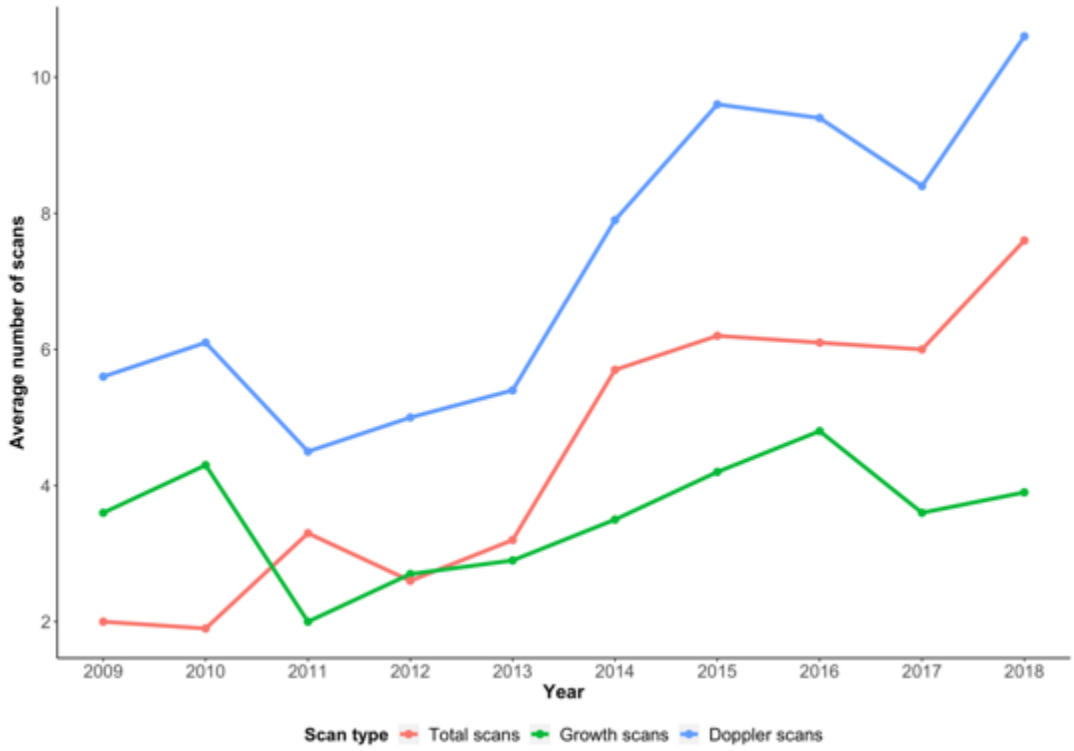


Figure 4.2: Change in the number of scans performed per eFGR patient over time.

To determine if this change over time has been significant, Poisson regression was used, adjusted for the first scan to delivery interval. For this portion of the analysis, only cases between 2012-2018 were included, as case numbers prior to 2012 were small. Cases were grouped by year as summarised in Table 4.3.

Table 4.3: Number of cases included in scan practice analysis according to year group.

Year group	Number of eFGR cases
1: 2012 – 2014	48
2: 2015 – 2016	56
3: 2017 - 2018	56
Total	*160*

The Poisson regression results are summarised in Table 4.4.

Table 4.4: Summary of Poisson regression to determine how scan surveillance has changed over time. Group 1: 2012-2014; Group 2: 2015-2016; Group 3: 2017-2018.

Time period comparison	Total number of scans		Number of growth scans		Number of Doppler scans	
	IRR (95% CI)	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*
Group 1 → Group 2	1.49 (1.30-1.72)	< 0.001	1.40 (1.14-1.71)	0.001	1.50 (1.24-1.82)	< 0.001
Group 1 → Group 3	1.27 (1.10-1.47)	0.001	1.01 (0.82-1.25)	0.941	1.49 (1.22-1.81)	< 0.001
Group 2 → Group 3	0.86 (0.76-0.97)	0.012	0.72 (0.60-0.87)	0.001	0.99 (0.84-1.17)	0.901

The total number of scans performed, adjusted for the length of time between the scan at diagnosis and delivery, increased to 2015-2016, then dropped slightly. Further analysis of the type of scans performed has shown that the delivery-interval adjusted number of growth scans increased to 2015- 2016, but there was a significant reduction in growth scans in 2017-2018, and the number of growth scans performed in this time period was comparable to 2012-2014. In terms of Doppler scans, when adjusted for the delivery interval the number increased to 2015-2016 but has remained constant since then. Overall, even when adjusting for the interval between the first scan and delivery, more scans are being performed in the eFGR cohort now compared to 7 years ago, and this is largely due to an increase in the number of Doppler scans.

4.3.1.3. Diagnosis to delivery interval / absent EDF to delivery interval

Table 4.5 and Figure 4.3 summarise the first scan to delivery interval.

Table 4.5: Yearly summary of interval from first scan to delivery in eFGR cohort. Data from 2009-2011 are included but shaded grey and not included in the analysis due to the small number of cases.

Year	Mean interval from first scan to delivery (days)
2009	25.2
2010	24.9
2011	23.4
2012	30.9
2013	21.3
2014	22.3
2015	26.4
2016	28.0
2017	26.0
2018	29.2

The pattern over time was analysed using linear regression and adjusting for the gestation at the time of the first scan. Cases which ended in FDIU were excluded from this analysis, leaving 122 cases included between 2012-2018. Table 4.6 summarises the results of the regression.

Table 4.6: Change in first scan-delivery interval over time, compared using linear regression. Group 1: 2012-2014; Group 2: 2015-2016; Group 3: 2017-2018.

Time period comparison	Coefficient (95% confidence interval)	P value
Group 1 → Group 2	4.09 (– 2.05-10.2)	0.190
Group 1 → Group 3	9.19 (3.23-15.2)	0.003
Group 2 → Group 3	5.10 (– 0.745-10.9)	0.087

Figure 4.3 shows the yearly change from 2012-2018. Since 2012 there has been an increase in adjusted scan to delivery interval of 9.19 days. Repeating this analysis excluding those cases that were clearly delivered due to a purely maternal rather than fetal indication (n = 17) does not change the pattern of results.

This analysis was repeated to determine how the interval from the first recorded episode of absent EDF has changed over time (Table 4.7), when adjusted for the gestational age at the first episode of absent EDF. The yearly change is depicted by Figure 4.3.

Table 4.7: Change in interval from absent EDF to delivery interval over time using linear regression. Group 1: 2012-2014; Group 2: 2015-2016; Group 3: 2017-2018.

Time period comparison	Coefficient (95% confidence interval)	P value
Group 1 → Group 2	4.08 (– 1.48-9.63)	0.149
Group 1 → Group 3	8.39 (3.14-13.6)	0.002
Group 2 → Group 3	4.31 (– 1.03-9.65)	0.112

Since 2012, the interval between the first recorded episode of absent EDF and delivery has increased by over one week (8.39 days; 95% CI (3.14-13.60)). This is not associated with significant changes in pregnancy outcomes.

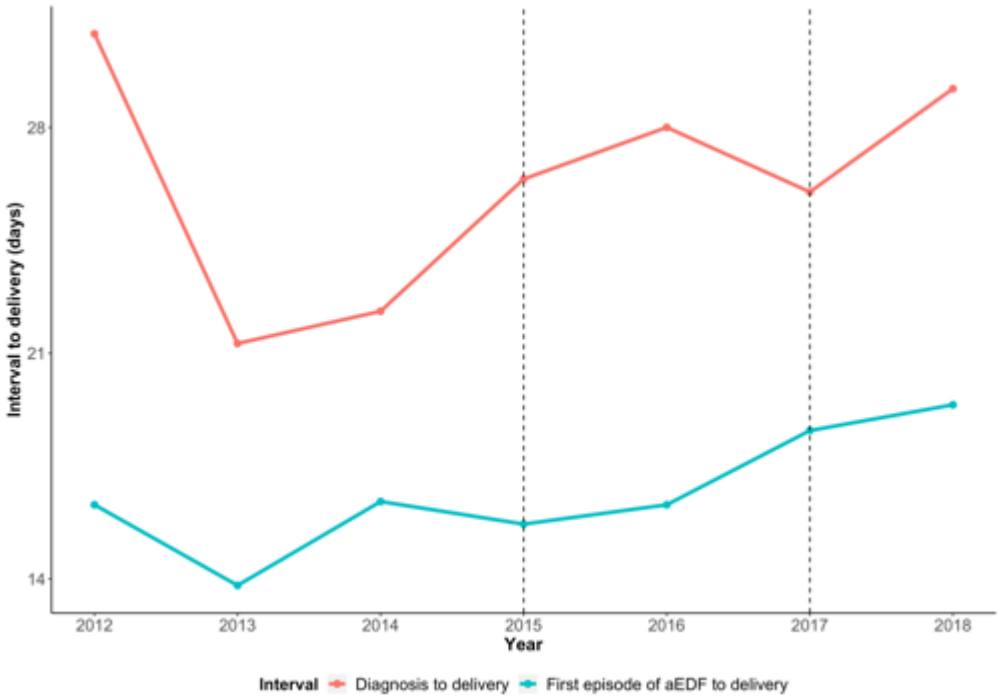


Figure 4.3: Change in interval between diagnosis of eFGR (red line) and first episode of absent EDF (blue line) and delivery. Dashed lines show how cohort is divided for purposes of data analysis.

4.3.1.4. Change in outcomes

Figure 4.4 shows the change in annual FDIU, neonatal/infant death, and overall death rates over time.

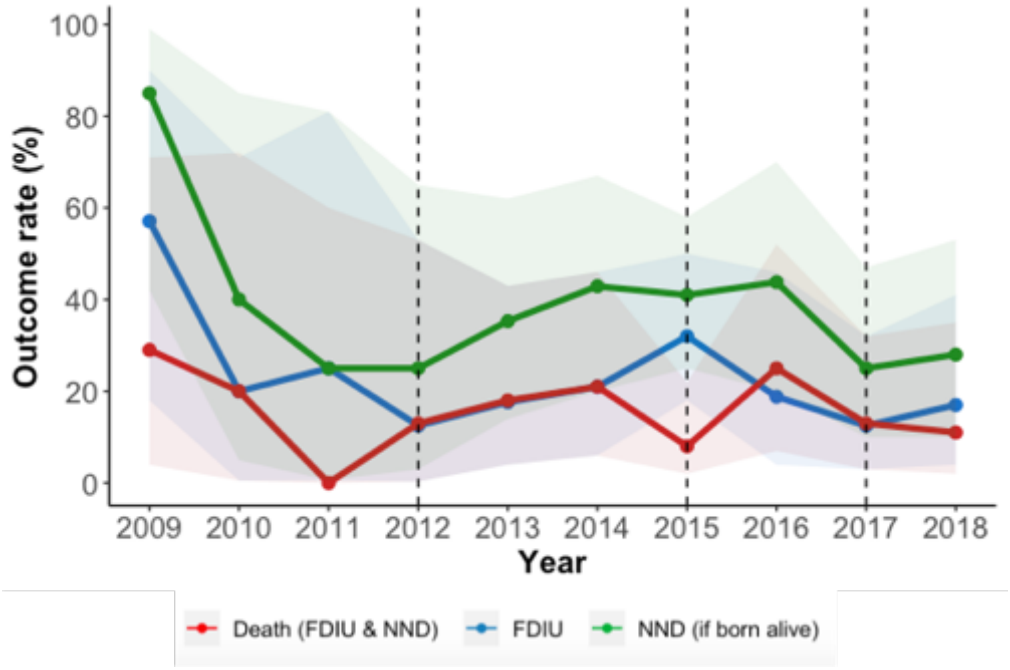


Figure 4.4: Change in FDIU / neonatal or infant death / overall death rates from 2009-2018.

Table 4.8 summarises the change in death rates over the period from 2012-2018. The overall death rate rose by 9% from 2012-2014 to 2015-2016; this was due to a 35% increase in the FDIU rate (P < 0.001), but a 22% decrease in the rate of neonatal death (P < 0.001). There was no significant reduction in neonatal death rate from 2016-2016 to 2017-2018 (P = 0.33), but the FDIU rate fell by 44% (P < 0.001). Overall death rates fell by over 30% from 2012-2014 to 2018-2019 (P < 0.001). These data do not allow any analysis of long-term neonatal outcomes.

Table 4.8: Change in the rate of FDIU / neonatal or infant death / overall death over the study period, as modelled by Poisson regression. Group 1: 2012-2014; Group 2: 2015-2016; Group 3: 2017-2018.

Time period comparison	**FDIU rate**		**Neonatal / infant death rate**		**Overall death rate**	
	IRR (95% CI)	*P value*	*IRR (95% CI)*	*P value*	*IRR (95% CI)*	*P value*
Group 1 → Group 2	1.35 (1.25-1.45)	< 0.001	0.78 (0.71-0.86)	< 0.001	1.09 (1.03-1.16)	0.01
Group 1 → Group 3	0.75 (0.68-0.82)	< 0.001	0.74 (0.67-0.82)	< 0.001	0.76 (0.71-0.82)	< 0.001
Group 2 → Group 3	0.56 (0.51-0.61)	< 0.001	0.95 (0.86-1.05)	0.33	0.70 (0.66-0.75)	< 0.001

4.3.2. Neonatal length of stay

A subset of the St Mary's data used for the survival analysis discussed in Chapter 2 was used to investigate the relationship between neonatal LoS and gestational age at delivery and birthweight in both eFGR and non-eFGR cases. For this analysis, only those cases ending in a live birth were used. Figure 4.5 summarises how this cohort was derived.

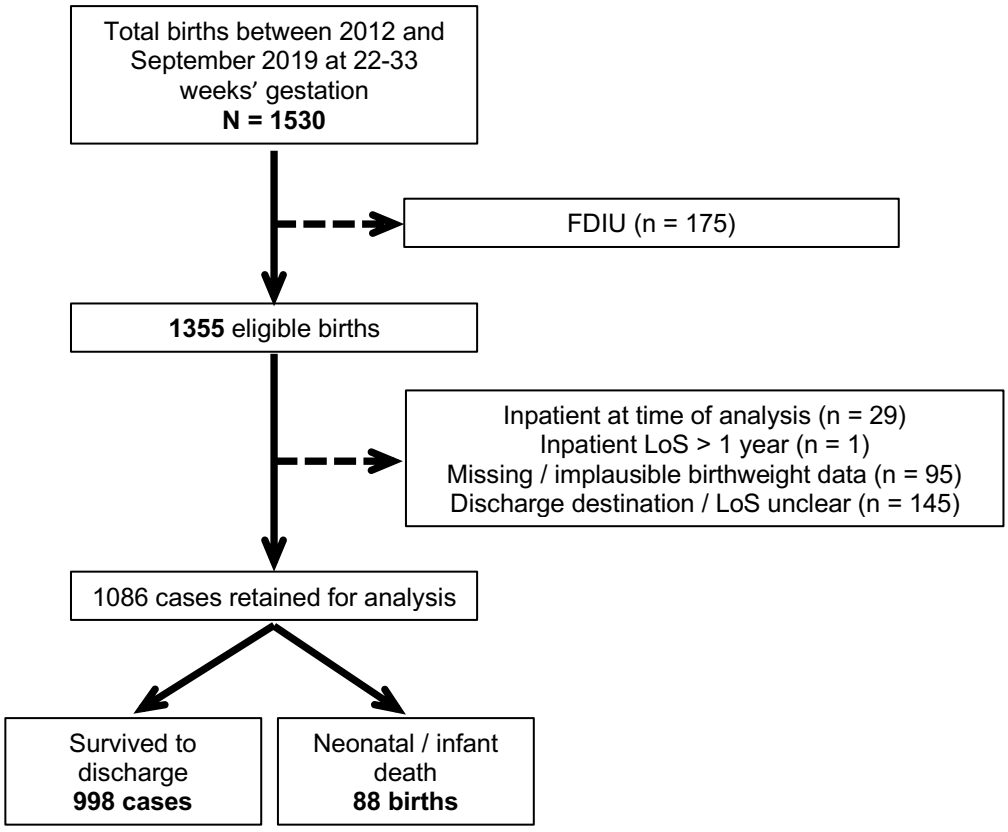


Figure 4.5: Flow chart to illustrate how final cohort for neonatal LoS was derived.

Pregnancy outcome data for this cohort are summarised in Table 4.9. Overall, 8.1% of infants born alive died before one year of age; as expected this proportion was significantly higher in those infants that were eFGR (15.4%) compared to those that were not (6.4%). eFGR cases were on average approximately 500g smaller than their non-eFGR counterparts in terms of birthweight ($P < 0.001$), although the gestational age at delivery was not significantly different (30.7 weeks in eFGR compared to 31.4 weeks in non-eFGR; $P = 0.77$).

Table 4.9: Summary characteristics of singleton live births at 22-33 weeks' gestation between 2012-September 2019 included in the length of stay analysis

	Whole cohort (n = 1086)	**> 3rd centile (n = 884)**	**≤ 3rd centile (n = 202)**	**Significance**
Gestation at delivery (weeks)*	31.1 (22.1-33.9)	31.4 (22.1-33.9)	30.7 (24.4-33.9)	P = 0.77
Birthweight (g)*	1441 (360-2746)	1590 (403-2746)	997 (360-1618)	
Outcome^				
Survived to discharge	998 (91.9%)	827 (93.6%)	171 (84.7%)	P < 0.001
Neonatal death	88 (8.1%)	57 (6.4%)	31 (15.4%)	
Infant sex^				
Male	488 (44.9%)	391 (44.2%)	97 (48.0%)	P = 0.33
Female	598 (55.1%)	493 (55.8%)	105 (52.0%)	

*Median (range); ^n (%)

The median LoS was estimated by pregnancy outcome, and across the whole cohort was 38 days (range 2-365). When broken down by eFGR status, eFGR babies had a significantly longer neonatal LoS of 52 days (range: 5-365) compared to 33 days in non-eFGR cases (range: 5-270) (P < 0.001; Mann-Whitney). This was then analysed by gestational age at delivery, with the gestational age split into 2-week groups. For a baby born at 24-26 weeks' gestation, the median neonatal LoS was estimated at 108 days (range 31- 270), which corresponds to a corrected gestational age at discharge of 40.4 weeks. As gestation at delivery advances, the neonatal LoS decreases and infants tend to be discharged ahead of their due date. For those born after 32 weeks, median LoS was 21 days, corresponding to a corrected gestation of 36 weeks. These data are summarised in Table 4.10.

Table 4.10: Summary of neonatal LoS according to gestational age for the whole St Mary's pre-33 week delivery cohort

Gestational age at delivery (weeks)	**Total number of cases**	**LoS of neonatal stay (median (range))**	**Days from midpoint of gestational age group to due date**	**Corrected gestational age at discharge from midpoint of gestational age group (weeks)**
22^{+0}-23^{+6}	14	127 (89-243)	119	41.1
24^{+0}-25^{+6}	86	108 (31-270)	105	40.4
26^{+0} – 27	98	84 (29-223)	91	39.0
28^{+0}-29^{+6}	147	59 (14-178)	77	37.4
30^{+0}-31^{+6}	222	37 (3-233)	63	36.3
≥ 32^{+0}	431	21 (2-184)	49	36.0

If the data are re-analysed according to whether or not the case was classified as eFGR, the pattern of results is different. These data are summarised in Table 4.11. For deliveries prior to 28 weeks' gestation, the LoS for both eFGR and non-eFGR infants corresponds approximately to the original due date, and there is no significant difference between eFGR and non-eFGR cases. However, after this gestation the LoS for eFGR infants is significantly longer, and even for those infants delivered after 32 weeks', the discharge date is at a corrected gestation of 37.1 weeks', compared to 35.9 weeks for non-eFGR infants (P < 0.001).

Table 4.11: Neonatal LoS according to gestational age at delivery and eFGR status. LoS data are displayed as median (range). Comparison made between LoS in non-eFGR and eFGR cases by gestational age using Mann-Whitney U test.

Gestational age at delivery (weeks)	Days from midpoint of gestational age group to due date	Non-eFGR			eFGR			Significance (non-eFGR compared to eFGR)
		Total number of cases	Neonatal LoS (days)	Corrected gestational age at discharge from midpoint of gestational age group (weeks)	Total number of cases	Neonatal LoS (days)	Corrected gestational age at discharge from midpoint of gestational age group (weeks)	
22^{+0}-23^{+6}	119	14	127 (89-243)	41.1	0			
24^{+0}-25^{+6}	105	83	106 (31-270)	40.1	3	174 (107-257)	49.9	ns (P = 0.07)
26^{+0}-27^{+6}	91	85	82 (29-223)	38.7	13	98 (58-149)	41.0	ns (P = 0.14)
28^{+0}-29^{+6}	77	110	55 (14-178)	36.9	37	74 (36-159)	39.6	P < 0.001
30^{+0}-31^{+6}	63	165	34 (3-95)	35.9	57	54 (24-233)	38.7	P < 0.001
$\geq 32^{+0}$	49	370	20 (2-184)	35.9	61	29 (5-78)	37.1	P < 0.001

4.3.2.1. Time to death

The time to death was also analysed in those that died before discharge, although these results should be interpreted with caution due to the low event rate (n = 88 neonatal / infant deaths). Data are summarised in Table 4.12. Across the whole cohort the median was estimated at 10 days (range 0-193), meaning that approximately half of deaths occurred in the first 10 days of life. Looking at these data by gestational age at delivery suggests that the time to death remains relatively constant at 10 days, until the delivery gestation reaches 32 weeks, at which point the median time to death increases to 25 days, although this is based on seven infants. Considering time to death by eFGR status, there is a larger spread of LoS in the eFGR cohort, which likely accounts for the trend towards a significant difference between the two groups overall (P = 0.05), but the median LoS in the non-eFGR cohort was 10 days, compared to just 11 days in the eFGR cohort. Numbers are too small to permit an accurate comparison by gestational age bin for eFGR and non-eFGR infants.

Table 4.12: Interval from delivery to death in those cases that ended in neonatal / infant death. Data are displayed first as the whole cohort, then split according to eFGR status.

	Whole cohort		Non-eFGR		eFGR	
Gestational age at delivery (weeks)	**Total number of cases**	**Time to death (days)**	**Total number of cases**	**Time to death (days)**	**Total number of cases**	**Time to death (days)**
22^{+0}-23^{+6}	17	10 (0-98)	17	10 (0-98)	0	
24^{+0}-25^{+6}	16	14 (0-63)	14	14 (0-63)	2	11 (2-19)
26^{+0}-27^{+6}	24	10 (0-193)	17	6 (0-103)	7	12 (2-193)
28^{+0}-29^{+6}	15	9 (0-42)	5	2 (1-15)	10	10 (0-42)
30^{+0}-31^{+6}	9	10 (5-97)	2	10 (5-15)	7	10 (6-97)
$\geq 32^{+0}$	7	25 (7-105)	2	41 25-56)	5	13 (7-105)

4.3.2.2. Effect of gestational age as a continuous variable on neonatal LoS

There is a significant relationship between neonatal LoS in those infants surviving to discharge and the gestational age at delivery. Using gestational age as a continuous variable, LoS is best modelled using a second order polynomial. With increasing gestational age, predicted neonatal LoS decreases, but an increase in gestation of one week at a later gestation has less of an effect on LoS than the same unit increase earlier on in gestation. The interaction between gestational age and eFGR status is not significant (P = 0.91), suggesting that the relationship between gestational age at delivery and LoS status is the same whether the infant is eFGR or not, but on average eFGR infants stay an extra 17 days in the neonatal unit compared to a non-eFGR infant born at the same gestation (P < 0.001). This relationship is summarised in Table 4.13 and Figure 4.6, which shows that although the curve of the regression line remains the same throughout gestation, LoS is higher in eFGR.

Table 4.13: Regression analysis to investigate the relationship between gestational age at delivery (weeks) and neonatal LoS (days), with inclusion of eFGR as a significant covariate.

Variable	Coefficient (95% CI)	P value
Gestational age (weeks)	– 45.49 (56.72- – 34.26)	< 0.001
Gestational age (weeks)^2	0.58 (0.39-0.77)	< 0.001
eFGR status Non-eFGR eFGR	 Reference 16.60 (12.75-20.45)	 < 0.001
Constant	889.01 (725.13-1052.89)	< 0.001

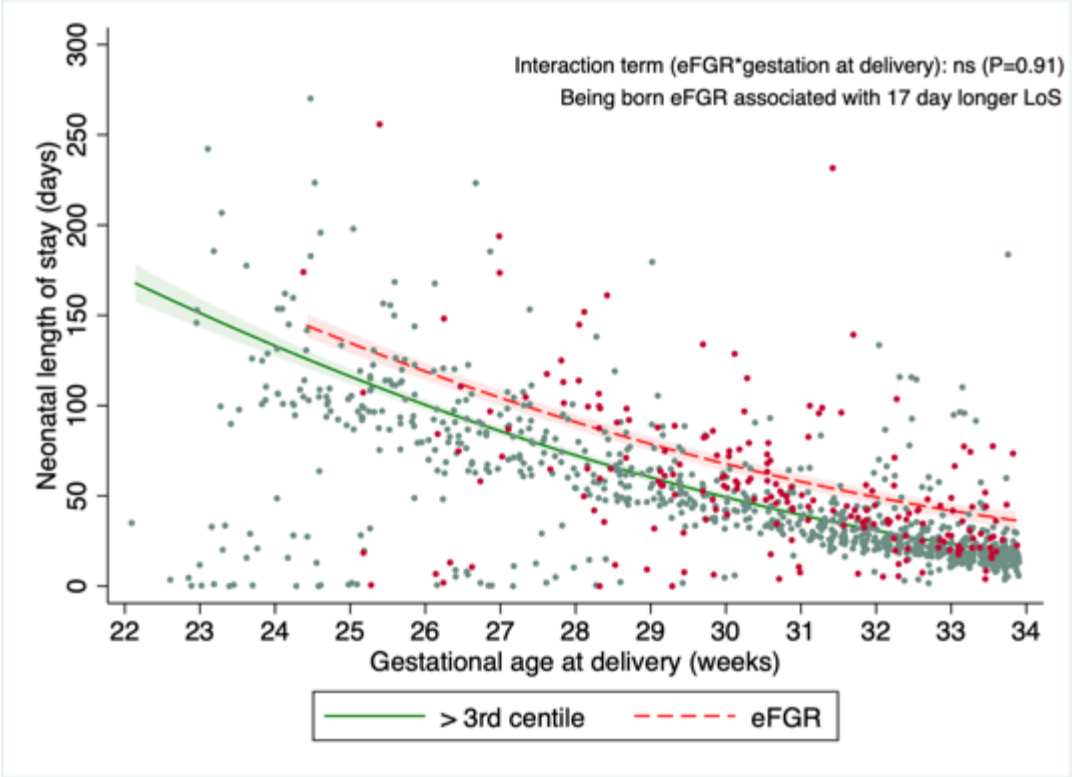


Figure 4.6: Scatter graph with regression line to show how gestational age at delivery as a continuous variable impacts on neonatal LoS in both eFGR and non-eFGR cases. The relationship between the two variables is not affected by eFGR status (non-significant interaction term P =0.91), but on average, eFGR infants tend to have a longer LoS by approximately 17 days (P < 0.001).

4.3.2.3. Effect of birthweight on neonatal LoS

Birthweight has a significant impact on neonatal LoS. Using birthweight as a continuous predictor, again LoS is best modelled using a second order polynomial, and as birthweight increases, the neonatal LoS decreases. As with gestational age, the interaction between birthweight and eFGR status is not significant (P = 0.30), but eFGR infants have a LoS 22 days shorter than an infant born at the same birthweight which was not eFGR (and therefore likely to be at an earlier gestation) (P < 0.001). This suggests that the gestation at delivery has a greater influence on LoS than birthweight in this cohort (Table 4.14; Figure 4.7).

Table 4.14: Regression analysis to investigate the relationship between birthweight (g) and neonatal LoS (days), with inclusion of eFGR as a significant covariate.

Variable	Coefficient (95% CI)	P value
Birthweight (g)	– 0.18 (–0.19- – 0.16)	< 0.001
Birthweight $(g)^2$	0.00004 (0.00003-0.00004)	< 0.001
eFGR status Non-eFGR eFGR	 Reference – 21.55 (– 25.77- –17.34)	 < 0.001
Constant	220.11 (208.57-231.65)	< 0.001

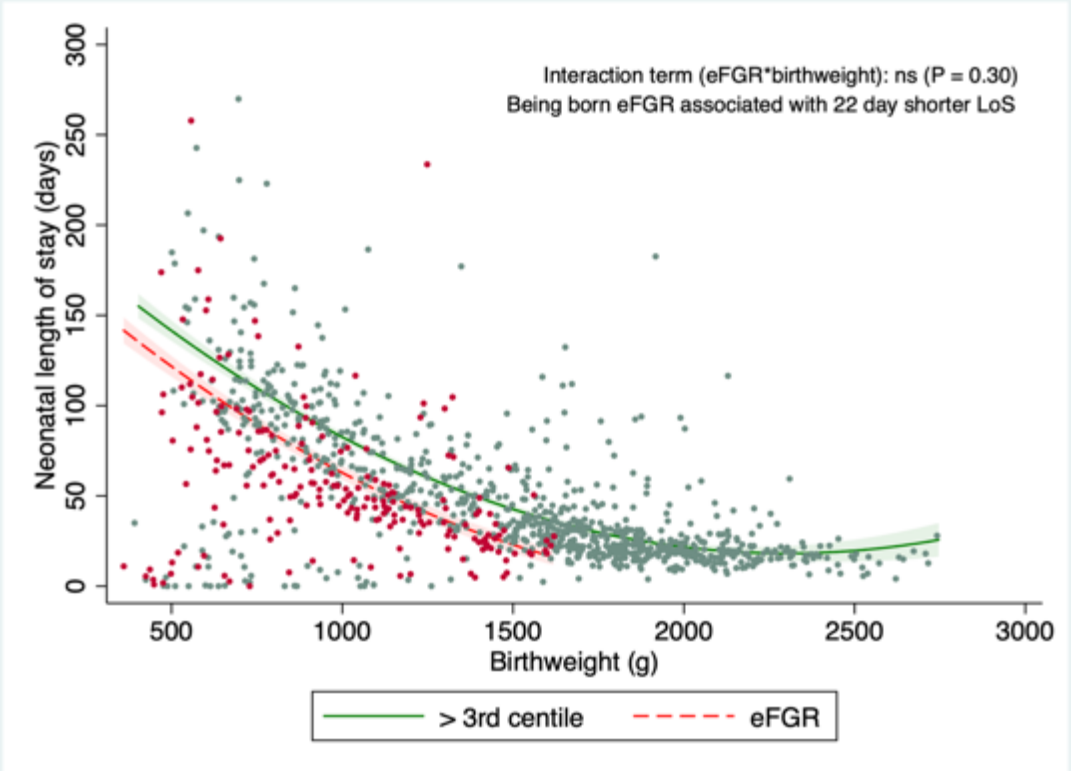


Figure 4.7: Scatter graph with regression line to show how birthweight at delivery as a continuous variable impacts on neonatal LoS in both eFGR and non-eFGR cases. The relationship between the two variables is not affected by eFGR status (non-significant interaction term P =0.30), but on average, eFGR infants tend to have a shorter LoS by approximately 22 days (P < 0.001).

4.3.2.4. Change in neonatal LoS over study period

There has been no significant change in the length of neonatal LoS over the period studied, in terms of the cohort as a whole, or when split by eFGR status. Results from the linear regression are summarised in Table 4.15. There is a trend towards a decrease in neonatal LoS over the time period for the whole cohort, but this does not reach significance (P = 0.09). When the cohort is split according to eFGR status, there does appear to be a significant decrease in the LoS between 2015-206 and 2017-2018 of 17 days (P = 0.03), however when the data are displayed graphically (Figure 4.8), it appears that this may be affected by skewed values. The analysis was therefore repeated with any outliers more than 2 standard deviations from the mean removed from the cohort. Results for this analysis are summarised in Table 4.16.

Table 4.15: Linear regression to investigate the change in neonatal LoS over the study period

Time period comparison	Coefficient (95% CI)	P value
Whole cohort (n = 1088)		
Group 1 → Group 2	–3.33 (–9.01-2.34)	NS (P = 0.25)
Group 1 → Group 3	–5.03 (–10.85-0.78)	NS (P =0.09)
Group 2 → Group 3	–1.70 (–7.86-4.46)	NS (P= 0.59)
>3rd centile infants (n = 885)		
Group 1 → Group 2	–5.34 (–11.49-0.81)	NS (P = 0.09)
Group 1 → Group 3	–3.42 (–9.70-2.86)	NS (P = 0.29)
Group 2 → Group 3	1.92 (–4.69-8.53)	NS (P =0.57)
eFGR infants (n = 203)		
Group 1 → Group 2	6.14 (–7.84-20.12)	NS (P =0.39)
Group 1 → Group 3	–11.27 (–26.00-3.45)	NS (P =0.13)
Group 2 → Group 3	–17.4 (–33.27- –1.56)	* P =0.03

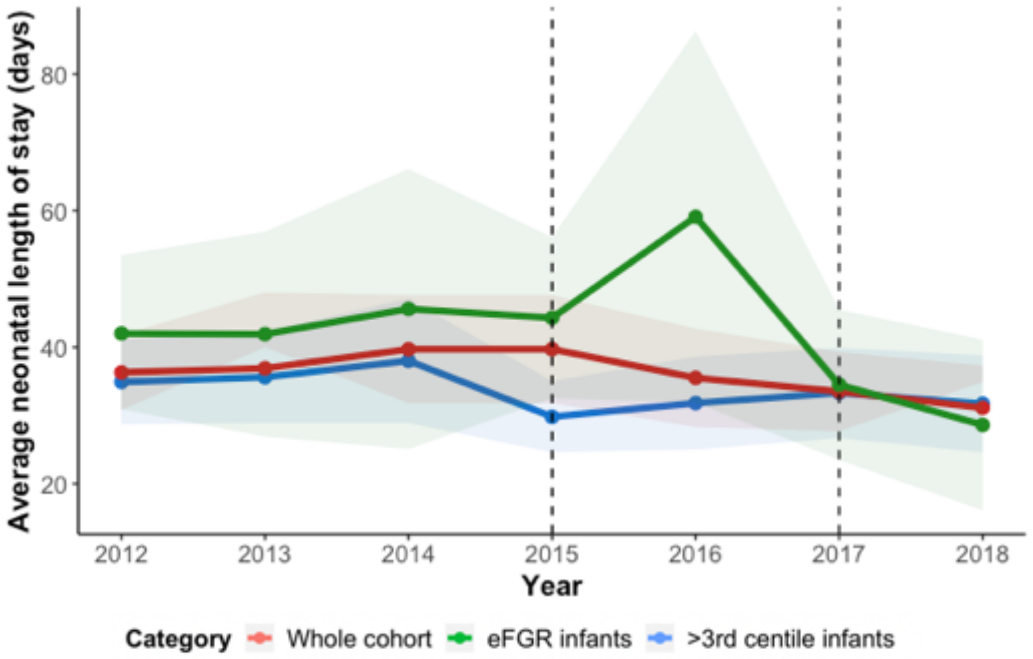


Figure 4.8: Average neonatal LoS for each year in the study period for the whole cohort (n = 1088, red), >3rd centile infants (n = 885, blue), eFGR infants (n = 203, green). Dotted lines represent how year groups were pooled for analysis (2012-2014 n = 398; 2015-2016 n = 309; 2018-2018 n = 283).

Table 4.16: Linear regression to investigate the change in neonatal LoS over the study period with outlier values >2 standard deviations from the mean removed from the analysis.

Time period comparison	Coefficient (95% CI)	P value
Whole cohort (n = 937)		
Group 1 → Group 2	– 1.64 (– 4.67-1.40)	0.29
Group 1 → Group 3	– 3.69 (– 6.80- – 0.57)	0.02
Group 2 → Group 3	– 2.05 (– 5.33-1.23)	0.22
>3rd centile infants (n = 767)		
Group 1 → Group 2	– 1.13 (– 4.42-2.16)	0.50
Group 1 → Group 3	– 3.43 (– 6.83- – 0.02)	0.048
Group 2 → Group 3	– 2.29 (– 5.82-1.23)	0.21
eFGR infants (n = 170)		
Group 1 → Group 2	– 2.05 (– 9.63-5.52)	0.60
Group 1 → Group 3	– 3.76 (– 11.23-3.70)	0.32
Group 2 → Group 3	– 1.71 (– 10.09-6.67)	0.69

Removing the outliers did not change the results of the analysis, therefore it can be concluded that over the study period of 2012-2018, there has been no significant change in neonatal LoS, regardless of eFGR status.

4.4. Discussion

4.4.1. Change in practice

The findings from this work suggest that over time the number of eFGR cases managed at St Mary's Hospital has increased, with an increase in scan surveillance, particularly in the use of Doppler ultrasound scans. There has also been an increase in the interval between both diagnosis and delivery, and first episode of absent EDF and delivery.

The increase in eFGR cases over time is likely influenced by a number of factors. Firstly, the steady increase in the number of cases from 2009 likely reflects the increasing awareness of the Placenta Clinic service, since its inception in 2009. This clinic is a regional service, and now receives referrals from all over the North-West of England. Secondly, the spike in numbers in 2015 coincides with recruitment to the STRIDER study (14), which took place between November 2014 and July 2016. Following 2015, the annual number of cases has fluctuated, but it remains to be seen if this reaches a relatively steady state. Other local units are in the process of setting up similar fetal growth clinics; however, in order to manage an eFGR case, they need to have access to specialist fetal Doppler scans seven days per week, and appropriate provision of neonatal care for delivery. This means that in the North-West of England, there are few units that are suitably equipped to deal with such cases from the time of diagnosis up until the point of requiring delivery. The incidence rates in Chapter 2 confirm that the total rates of eFGR remain relatively constant over time, therefore it would be expected that the number of cases seen in the Manchester Placenta Clinic would also reach a steady state.

In terms of antenatal scan surveillance, more scans are performed in the eFGR cohort now compared to seven years ago, even when adjusted for the interval between the first scan and delivery. This is largely related to an increase in the number of Doppler scans. The TRUFFLE study was published in 2015, and advocated the use of the DV Doppler as a trigger for delivery (76), which will have contributed to the increase in Doppler scans performed after this time. In addition, the STRIDER protocol dictated that growth scans be performed weekly, which will have led to an increase in growth scans at the time of the study (14). Standard practice in this unit is to perform growth scans every fortnight, therefore accounting for the subsequent reduction in the IRR when comparing 2015-2016 to 2017-2018 (0.72, 95% CI 0.60-0.87); $P < 0.001$). Performing more scans per patient will obviously have a cost implication, with the cost of an antenatal ultrasound scan being £53 (226). However, if this increased surveillance results in a prolonged gestation then overall it is likely to result in savings, as the national average cost of a day's care on the neonatal unit is £1531 (226). This does not consider the long-term economic implications of the costs associated with severe neonatal morbidity, which can extend over childhood and beyond. Increased surveillance should also lead to fewer emergency deliveries and fewer women who do not have time to receive antenatal corticosteroids and magnesium sulphate which further reduce neonatal mortality and morbidity (227,228). Regular scans will enable delivery to be arranged at the optimum time, prior to decompensation necessitating immediate delivery, and this will result in optimal administration of steroids and magnesium sulphate, better condition of the baby at delivery,

improved allocation of neonatal resources with advanced planning and less emotional distress for the family.

Extending the pregnancy by an average of 9.19 days compared to 2012 possibly represents increasing confidence and knowledge in antenatal surveillance in eFGR. This, coupled with the increase in the use of Doppler scans to monitor patients, suggests that over time we are becoming more reliant on changes in Doppler parameters to indicate delivery. A similar pattern is seen in the interval between the first episode of absent EDF and delivery, which has increased by over one week since 2012 (8.39 days (95% CI 3.14-13.6); P = 0.002). This has been associated with a reduction in overall death rates over the study period. Due to the perceived increased stress that occurs when a fetus has abnormal EDF, prolonging this interval could be associated with a higher incidence of neonatal complications. However, the evidence surrounding this is unclear, with some studies suggesting an increased likelihood of NEC (229,230) and cerebral haemorrhage and other studies unable to corroborate this finding (231). Overall, there are few studies that directly address this question, indicating that there is a need for this area to be explored. This is particularly important as it seems that we are now prolonging pregnancies in cases with absent/reversed EDF with the belief that this gain in gestational age is beneficial for neonatal survival, but if there is evidence that this could increase the risk of certain complications then this practice would need to be reconsidered. There are however a number of confounding factors involved, including extreme prematurity, extremely low birthweight, other Doppler parameters and maternal disease. All of these factors are linked, and will all influence the risk of neonatal morbidity, therefore it is unlikely that absent EDF alone would be the contributing factor to development of complications such as NEC and cerebral haemorrhage.

This dataset is too small to observe any meaningful changes in different pregnancy outcome rates, although a significant reduction in overall death was seen and outcome rates are similar to those reported by the STRIDER study (15). It would be hoped that over time as management practices adapt to new evidence and neonatal care continues to improve, then the rates of FDIU and neonatal death would decrease, as the data presented here suggest. In addition, long-term neonatal outcomes are not examined at all in this study. Further information regarding this in the context of early-onset FGR should be available in the future from both the TRUFFLE and STRIDER studies.

4.4.2. Neonatal length of stay

These data have confirmed that St Mary's Hospital is in line with UK-wide data regarding the neonatal LoS for infants born prior to 33 weeks' gestation. It has added to this by showing that there are important differences to consider between the neonatal LoS in those infants affected by eFGR compared to those that are not.

Seaton et al. (232) recently published results of a national study to predict neonatal LoS in singleton preterm babies. Although different statistical methods of comparison were used, similar overall conclusions are drawn albeit on a much larger scale. The neonatal death rate in this population was 8.6%, compared to 8.1% in St Mary's Hospital, and as was concluded from the St

Mary's study, around half of all neonatal deaths took place in the first 10 days of life (232). They also concluded that infants born at the earliest gestations (pre-26 weeks') are discharged around their original due date, but that those born beyond 30 weeks' gestation tend to be discharged four weeks prior to the due date (232). These findings corroborate the conclusions from the St Mary's dataset presented in Chapter 3.

Unsurprisingly, being eFGR is associated with having a significantly longer stay on the neonatal unit, regardless of the gestational age at delivery. Data presented previously in this thesis have confirmed the high rates of neonatal mortality and morbidity that have been demonstrated in other studies of extremely growth restricted fetuses (11,15,233), however this study provides an estimate of the additional time that eFGR infants require in neonatal care.

At first glance, the relationship between birthweight, eFGR status and neonatal LoS appeared unexpected, with eFGR infants born at a given birthweight discharged 3 weeks earlier than their appropriately grown counterparts. Logically, however, this finding fits with the nature of eFGR; an infant with a birthweight of 800g that is classed as eFGR is likely to be born at a gestation of 28 weeks' at the earliest, compared to an infant born weighing 800g on the 50th centile, which would be born at 25-26 weeks'. An additional research question that would provide more information regarding this relationship between gestational age, birthweight and LoS would be to determine the weight that an infant reaches at the time of discharge / time of decision to discharge. This would allow us to determine whether it is absolute age or weight gain or weight gain trajectory that appears to be the driving force behind the infant reaching a condition that is suitable for discharge.

There does not appear to have been a significant change in the length of neonatal stay over the study period. With the increase in the interval from diagnosis and first presentation of absent EDF to delivery, overall costs will be higher as the antenatal period is longer and more scans are performed for monitoring purposes with no compensatory decrease in neonatal LoS. This increase in antenatal costs would be offset however if there was a change in long-term outcomes and the level of care needed in the future, which cannot be concluded from this study. Despite no appreciable change in the neonatal LoS, pregnancy outcomes in this cohort appear to be improving from the data presented here, which should outweigh any economic implications.

4.4.3. Strengths and limitations of the study

The data collected for this analysis come from a single tertiary care unit, so give an accurate picture of the practice specific to this unit and can be used to inform affected families of infants who will be cared for at this unit. As the data were all collected locally, it was possible for accuracy to be checked. The use of BadgerNet means that in the majority of cases, even those infants who were discharged to another unit could be included in the analysis as data regarding discharge date was still available from units that also use BadgerNet.

Previous studies have not included data on 22-24 week deliveries. The decision was made to retain these cases in this analysis; because data were collected from a single unit, individual case files could be accessed to confirm details and although attitudes to deliveries at these pre-viable gestations have changed over time, collecting data from just one unit reduces the variation in

management that will also occur between units. Finally, as previously highlighted, there are several consistencies between the data presented here and those presented by Seaton et al., namely in the similar neonatal death rate (8.6% nationally (232) compared to 8.1% at St Mary's Hospital), and similar LoS findings. This provides further encouragement that the conclusions drawn here are valid and may be generalisable to other eFGR populations in the UK.

The are several limitations to the data collected and the analysis performed. A proportion of the dataset was excluded due to missing neonatal discharge information or implausible birthweight data, which precluded the calculation of birthweight centiles and determination of eFGR status (n = 240; 18%). It may be that this has led to bias in the data, depending on the reason why the information was missing.

This analysis was not the primary objective for the study at the time of data collection. With retrospect, the data collection may have been performed differently, with additional information such as major co-morbidity or infant weight at discharge, which could have enabled a more comprehensive review of neonatal stay.

These data were also taken from a single unit, so is only reflective of the practice within this unit and conclusions should not be extrapolated to other level 3 NICU units. The characteristics of the population are similar to previous studies though, as highlighted in the paragraph above regarding study strengths.

Furthermore, this analysis does not account for the different levels of neonatal care, and the varying lengths of time infants with different co-morbidities spend at each level. There is a differing cost involved with neonatal ICU compared to neonatal high dependency unit (HDU) (226); it may be that eFGR infants spend longer at a higher level of care, which would have further economic implications. Finally, this study does not take into account the discharge policy at St Mary's Hospital. Individual units are likely to have their own guidance regarding key indicators, such as critical discharge weight, oxygen requirements and feeding requirements, which could have an additional impact on LoS in those infants transferred between different units.

4.4.4. Clinical implications

The conclusions drawn from these data provide figures that can be used when counselling parents affected by a preterm delivery. "How long will my baby stay?" is a commonly asked question in this scenario, and families are often advised to prepare for their baby to come home around its original due date, although this information is not evidence-based. These data show that this is not always the case, in line with conclusions drawn by previous studies (232,234,235), and provides estimates that take into account the gestational age, and also the birthweight, specific to St Mary's Hospital. Furthermore, this study has provided additional information within the context of eFGR, which no study to date has previously addressed.

From a health economic standpoint, this chapter as a whole provides a basis on which to begin to assess the economic impact of eFGR. Obviously the most influential statistics about eFGR are its associated mortality and long-term morbidity outcomes. However, in the current economic climate, where both healthcare resources and research budgets are finite and extensive resource planning

is required, having an awareness of not only the clinical but also the economic implications of management decisions in eFGR will influence future care in this area.

4.4.5. Future work

As highlighted in the previous section, this analysis is helpful within the context of analysing the practice of eFGR management and potential economic implications within the St Mary's population, but to draw any wider economic conclusions regarding the cost of eFGR would require data to be collected from a broader population. Data regarding neonatal LoS is available on a national level through the National Neonatal Research Database. Assessing change in practice on a population level would be difficult to replicate in other units due to the unique nature of the translational research clinics at St Mary's and the detailed database available for analysis.

A different statistical approach is to use a flexible parametric competing risks model as used by Seaton et al. (232) to investigate the neonatal LoS. This would take account of those infants that died whilst an inpatient, which has been previously highlighted as a limitation to this type of research (236). In addition, no previous studies have addressed the neonatal LoS question in a population of eFGR infants. Although the data presented here have started to address this, a population level study would provide much more robust answers. In addition, investigating the relationship between the primary neonatal problem (e.g., respiratory / gastrointestinal / neurological) and the LoS could reveal subsets of infants that are more prone to a prolonged stay or a faster recovery. Both these questions could potentially be answered through use of the National Neonatal Research Database, which makes use of the BadgerNet platform (237).

4.4.6. Conclusions

The analysis presented in this chapter has revealed that management of eFGR in this large tertiary unit has changed over time and attempted to address potential reasons for these changes. This provides a unique opportunity to reflect on eFGR management, and a chance to determine how the important studies in this area in the past years (namely TRUFFLE and STRIDER (15,76)) and the development of a specialist clinical service have shaped the management of eFGR on a local level.

Our findings regarding the neonatal LoS are in keeping with larger national studies, which can be taken as reassurance that management within St Mary's maternity and neonatal unit is largely in keeping with the country as a whole. It also provides information that can be given to parents in advance of a preterm delivery specific to the unit in which their baby will be cared for, which should help to alleviate some of the inevitable anxiety surrounding the postnatal period.

CHAPTER 5: ANTENATAL FETAL HEART RATE MONITORING IN eFGR

5.1. Introduction

Earlier in this thesis, antenatal sonographic prognostic factors were identified, measured both at the time of diagnosis and longitudinally as the pregnancy progresses, that can inform prognosis, but there remains a need for additional tools to improve this further.

Fetal heart rate monitoring forms an important part of antenatal surveillance in eFGR, even more so since the results of the TRUFFLE trial, which included monitoring STV as a trigger for delivery (238). Fetal heart rate monitoring provides real-time information reflecting fetal wellbeing, compared to ultrasound parameters which provide a more global overview reflective of placental function and fetal growth. Identifying a prognostic factor from the CTG that can be used in conjunction with ultrasound findings could strengthen the predictions that can be made about pregnancy outcome and provide further information to better time delivery. There have been no studies to date directly comparing cCTG in normal pregnancies to those affected by eFGR, but long-term fECG monitoring has shown promise as a valid method of monitoring SGA fetuses (defined as birthweight $\leq 10^{th}$ centile) in the home environment (239). Previous work has found that SGA fetuses have lower long- and short-term variability and fewer accelerations (240), and a lack of the diurnal variation seen in normally grown fetuses (241).

Due to the nature of the equipment used to record conventional Doppler CTG, the majority of CTG traces provide short (~1 hour) snapshots of the current fetal state. Use of the Monica AN24 device allows longer fECG recordings to be made, therefore providing a better chance of identifying any differences that may exist between normal and eFGR pregnancies (242). Analysis of standard cCTG parameters provides a clinically relevant comparison between normal and eFGR pregnancies, however the wealth of data that is available from a single Monica AN24 recording lends itself to alternative methods of FHR analysis, such as recurrence analysis or PRSA. PRSA has already been suggested as a method of monitoring progressive deterioration in early-onset FGR through a secondary analysis of the TRUFFLE data (171). This study concluded that AAC and ADC showed a statistically significant decrease from baseline to 1-5 days prior to delivery, and the authors suggested that this may therefore be a method of identifying chronic hypoxia and fetal decompensation earlier than using STV (171). This study did not specify the length of recordings used, and only used data where delivery / FDIU occurred within five days of the recording, whereas the present study proposes the use of a longer recording period, no limit to the interval between the recording gestation and delivery, and a comparison with gestation-matched control pregnancies.

This study aims to add to current knowledge by performing a direct comparison of cCTG parameters between eFGR and control pregnancies using a longer-term recording method than has previously been used. In addition, it aims to explore potential alternative methods of FHR analysis, with a view to determining if cCTG analysis can add additional prognostic data in the context of eFGR. Although not the first study to examine the cCTG in eFGR, it is the first study to look at FHR patterns in eFGR over an extended monitoring period.

5.2. Methods

5.2.1. Ethical approval

Ethical approval was obtained from the Greater Manchester Central North West Research Ethics Committee and the Manchester University NHS Foundation Trust R&D office (IRAS ID: 124307; REC reference: 15/NW/0829), and all work was conducted in accordance with the Declaration of Helsinki 1975 (revised 2013). Potential participants were approached by the study clinician and provided with written information about the research project prior to written confirmation for FHR monitoring being obtained.

5.2.2. Participants

Women were recruited from St Mary's Hospital, Manchester. Inclusion criteria were: singleton pregnancy, between 23^{+0} and 32^{+0} weeks' gestation. Exclusion criteria were: maternal age less than 16 years old, known fetal abnormality, multiple pregnancy and inability to provide informed consent.

5.2.2.1. Low risk participants

Low risk participants were defined as those without any evidence of FGR in their current pregnancy; these participants were used as the control group for this study.

5.2.2.2. eFGR participants

eFGR participants were those with a diagnosis of eFGR, defined as per the Delphi consensus (8):

- EFW/AC < 3rd centile
- EFW/AC 3rd-10th centile AND UA aEDF OR UtA PI > 95th centile for gestation.

5.2.3. Study protocol

5.2.3.1. Data collection

At recruitment, maternal demographic and antenatal booking data were recorded. Pregnancy outcome data were recorded following delivery. Variables collected are summarised in Table 5.1.

Table 5.1: Summary of study data collected for each patient recruited.

Patient demographics	Maternal age Booking height (cm) Booking weight (kg) Booking BMI (kg/m^2) Maternal ethnicity
Obstetric history	Booking parity Previous pregnancy complications
Recording demographics	Gestational age at recording (weeks+days) Gestational age at closest scan (weeks+days) (within seven days of monitoring) EFW at closest scan (g) (within seven days of monitoring) EFW centile at closest scan (within seven days of monitoring) UA Doppler at closest scan (normal / PI>95th centile / absent / reversed) (within seven days of monitoring) Gestational age at delivery (weeks+days) Birthweight (g) Birthweight centile Infant sex Pregnancy outcome (live born, still living / FDIU / neonatal or infant death) Date of steroid administration (if applicable)

5.2.3.2. Fetal heart rate monitoring set up

Fetal heart rate monitoring was performed using the Monica AN24 device (GE Healthcare, UK) (Figure 5.1(a)) according to the manufacturer's instructions. Firstly, the skin on the maternal abdomen was prepared using Red Dot Skin Prep Tape (3M, Bracknell, UK) by making a cross-like mark to remove dead skin cells and improve skin conductance. Secondly, five Blue Sensor VLC ECG electrodes (Ambu, Cambridge, UK) were applied in the formation shown in Figure 5.1(b). Thirdly, the electrode leads were connected to the Monica AN24 device and the device turned on. A Bluetooth connection between the device and a laptop computer running the Monica VS software (GE Healthcare, UK) was established, and the connection between the electrodes and the skin was checked. Once the signal strength was assessed as adequate, and there was no interference with any of the electrodes, heart rate monitoring began automatically. If the software highlighted a connection issue with any of the electrodes, the electrodes were replaced and the signal strength and interference re-assessed, until an adequate recording was obtained.

Once monitoring had begun, the trace was observed for a period of 2 minutes to ensure that both maternal and fetal heart rates were being recorded. Following this, the computer software was shut down, and the participant was allowed to return home with the device *in situ* still recording.

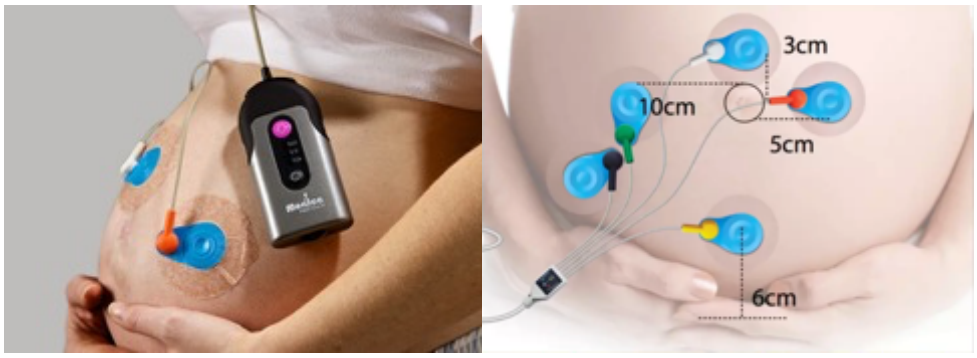


Figure 5.1: (a) Example of the Monica AN24 device; **(b)** Electrode placement on the maternal abdomen. Source: http://www.monicahealthcare.com/. Last accessed 13th Jan 2018.

5.2.3.3. *Fetal heart rate data download*

The participant was asked to remove the electrodes and return the device to the Maternal & Fetal Health Research Centre in St Mary's Hospital, Manchester approximately 24 hours after the recording was commenced, or when the battery on the device had run out. The device was then connected via USB cable to the laptop, and the Monica VS software used to visualise and download the data. An initial assessment of the CTG was made by a clinician and provided there were no immediate concerns with any features of the FHR, the participant returned home. If any concerning features were noted, immediate further antenatal review was arranged.

Two files were downloaded from the Monica VS software for each recording:

1) .csv file containing the time, maternal heart rate (bpm), FHR (bpm), maternal movements, signal and uterine activity;
2) .txt file containing summary data from the recording, including the amount of signal loss, and the Dawes-Redman criteria for each 60 minute recording period, as calculated by the Monica VS software.

5.2.3.4. *Sample size justification*

As a novel method of FHR analysis is being used in the context of eFGR, there are no existing studies on which to base a power calculation. Study power (power level 0.9, statistical significance $P<0.05$) was calculated from an interim analysis of 19 recordings (n = 9 low risk, n = 10 eFGR). This demonstrated a potential difference between the number of small accelerations per hour ($P = 0.06$) and large decelerations per hour ($P<0.05$). To relate the number of large decelerations per hour to outcome in eFGR, 30 eFGR pregnancies would need to be recruited, therefore this was set as the recruitment target.

5.2.3.5. *Statistical analysis*

The following FHR parameters were calculated by the Monica VS software, based on the Dawes-Redman criteria: mean FHR, basal FHR, small accelerations, large accelerations, small decelerations, large decelerations, short-term variation, mean minute range, high variation, low variation.

All statistical analysis was performed using Stata 15.1 for Mac (StataCorp, College Station, TX, USA) or R: A language and environment for statistical computing (176); figures were produced using the graphical package ggplot2 (194).

5.2.3.5.1. Basic comparison between low risk and eFGR groups

The Dawes-Redman criteria as calculated by the Monica VS software were compared between the two groups. Normality for each parameter was determined using the Shapiro-Wilk normality test. For each case a mean value across the whole recording was determined for each parameter. Using this value, each parameter was compared between low risk and eFGR patients. Data are expressed as mean $\pm$ SD or mean (range) as appropriate and displayed as box and whisker plots. Comparisons between the two groups were made using either a t-test or Mann-Whitney U test as appropriate.

Data were then split according to day or night time recording. Day time was defined as 0700-2159 and night time defined as 2200-0659. For each recording a mean value for each parameter was calculated for the day and the night time periods. Comparisons were then made between the two groups for day time readings and night time readings, using the same methods described above. No adjustments were made for uterine activity or maternal movement.

5.2.3.5.2. Longitudinal data analysis

Mixed level regression analysis was used to determine how cCTG changed throughout the day, and whether this differed depending on whether the recording was performed in a low risk or eFGR pregnancy.

For the fixed portion of the model, the Dawes-Redman parameter was included as the dependent variable, and hour as the independent variable, with low risk/eFGR status included as an interaction term. Patient ID was included in the random segment of the model, to account for the multiple time points within the same recording. Only the first recording for each patient was used for this section of the analysis.

5.2.3.5.3. Recurrence analysis

Given the potential enormous size of the dataset available from each Monica AN24 recording (e.g. a 20 hour trace with 25% signal loss will result in ~1,350,000 FHR measurements), novel methods of longitudinal analysis are required to maximise the information gained. Recurrence analysis is explored in this thesis as a method of longitudinal data analysis.

Recurrence analysis is a mathematical method of visualising the repetitive behaviour of dynamic systems, such as FHR variability, and turning a seemingly chaotic signal into a new ordered signal, which can reveal different physiological states. For example, such methods have been applied to the analysis of blood pressure waveforms in the investigation of sepsis in an animal model [personal communication – Hitesh Mistry, August 2017]. As illustrated by Figure 5.2, by using attractor reconstruction and Takens' vectors, the shape of the BP waveform can be quantified over time, and differing phase plane shapes can be seen as the sepsis progresses, whereas little difference is seen in the original BP waveform itself. This illustrates how recurrence analysis could

be a sensitive method of identifying decompensation earlier that it would usually be detected by monitoring conventional parameters, therefore potentially allowing earlier intervention.

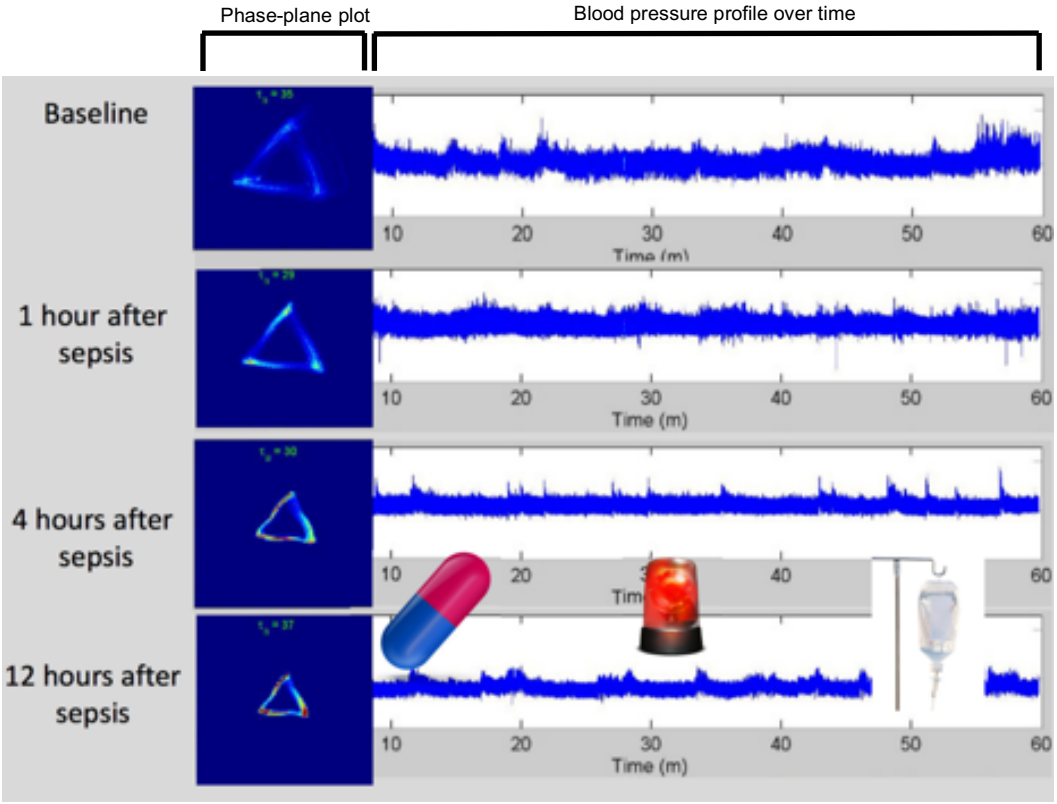


Figure 5.2: Example of attenuator reconstruction of the phase plane space applied to blood pressure monitoring in sepsis. Figure shows that there is little meaningful difference in blood pressure trace as sepsis progresses, but the shape of the phase plane plot is changing with time. Dr H. Mistry 2017, personal communication, August 2017.

In a recurrence plot, or reconstruction of the phase plane space, the recurrences of a dynamic system, such as the FHR, are plotted in phase space. Conventional monitoring provides a simple time series of observations, rather than the phase space. To create the phase space, Takens' vectors are used to embed the time series in *m*-dimensions with a set time lag, T, as depicted by Figure 5.3.

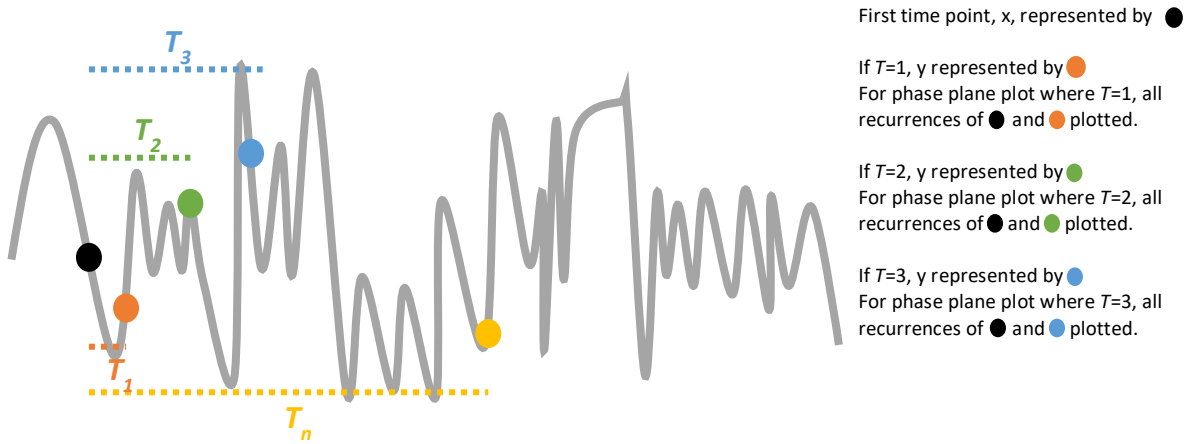


Figure 5.3: Schematic representation of Taken vector construction - the FHR is represented by the grey line, with individual time points represented by the coloured dots.

In the case of the FHR trace, phase plane plots are created in two dimensions. For a time lag of 0.25s, the FHR at a given time point is taken as *x* and the FHR 0.25s later (T_1) is taken as *y*, for

every FHR measurement in the recording, These vectors are then plotted to give the phase space in two dimensions. The time lag can be increased, for example by a factor of 10 to 2.5s, then to 25s etc., and the changing shape of the resulting phase plane plots can be compared between control and eFGR cases. Nonlinear analysis techniques have been applied previously in obstetrics to FHR recordings obtained through ST Analysis following acquisition of the fECG through a fetal scalp electrode, to show that such techniques were better than conventional monitoring at distinguishing between fetuses with and without acidaemia during labour (243).

Further analysis of the phase plane space can be undertaken using *k*-means clustering. This is a method of unsupervised learning to define groups within a dataset. Rather than defining the groups pre-analysis, which could be subject to bias, *k*-means allows groups to be determined organically by the data, and can uncover previously unseen groups in complex datasets (244). Each cluster is defined by a centroid, examination of which can qualitatively interpret what type of group the cluster represents; in the case of FHR data this may represent different physiological processes which could be related to pregnancy outcome.

The R package “Nonlinear Time Series Analysis” (245) was used to construct Takens’ vectors from the raw FHR data in two dimensions, with discrete time lags. FHR data were imported into R, and cleaned to remove any FHR measures recorded as zero, or readings where it matched the maternal HR. Takens’ vectors were calculated and plotted to show the changing phase plane space for each time lag used. Time lags calculated were 10, 20, 50, 100, 200 and 500 seconds. *K*-means clustering was then used to determine the number of clusters detected at each time lag, with a neighbour count of at least 50 within a radius of three used to define the core points.

5.3. Results

5.3.1. Recruitment

In total, 82 women were approached to take part in the study. 18 declined to participate (either due to lack of time, concern about effect on skin or fetus, or anxiety), leaving 65 women who underwent FHR monitoring. Of these, 31 were control cases and 34 were eFGR. One control case was excluded as no useable FHR data was recorded. Three eFGR cases were subsequently excluded, one due to late booking and ambiguity around the expected date of delivery (making the calculation of gestation inaccurate), and two as no useable FHR data were obtained. 11 of the participants with eFGR underwent a second episode of FHR recording, either after a change in Doppler parameters or after receiving antenatal corticosteroids. These data are summarised by Figure 5.4. Two cases were reclassified following collection of the pregnancy outcome data. This is because they met the eFGR criteria at the time of recruitment but continued to gain weight and had normal fetal Dopplers throughout pregnancy, and therefore did not require delivery until after 36 weeks' gestation. For the purposes of the analysis, these cases were reclassified into the control group. Each analysis was performed with and without these cases included in the control group, with no change in the conclusions drawn.

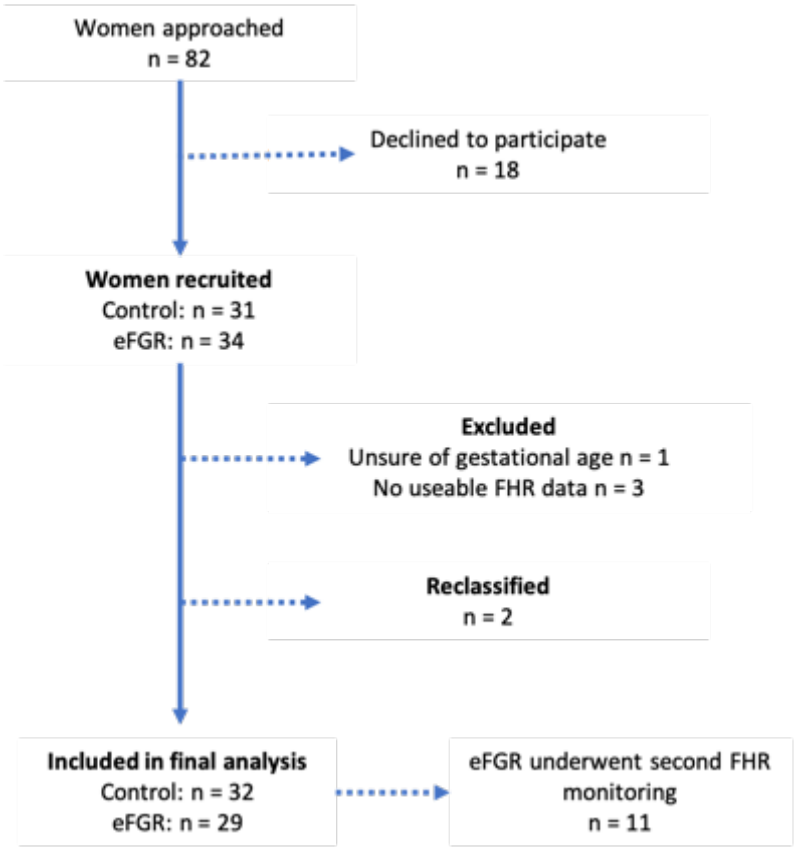


Figure 5.4: CONSORT diagram to summarise recruitment of patients to the Monica AN24 study.

5.3.2. Maternal demographics and pregnancy outcomes

Maternal demographics of the study population are summarised in Table 5.2. The two cohorts were similar in terms of maternal BMI and parity, but there was a significant difference in maternal age and in ethnicity. There was no difference in rates of previous pregnancy complications. A large number of the control recruits came from the Manchester Rainbow clinic, which runs within the Maternal & Fetal Health Research Group and is responsible for the care of women who have suffered a previous FDIU. Women recruited from this clinic had a previously unexplained FDIU or neonatal death (i.e., not related to recurrent conditions or placental disease), and were otherwise low risk in their current pregnancy. None of the differences between the two cohorts, however, would be expected to influence the FHR.

Table 5.2: Cohort characteristics for patients recruited for FHR monitoring, according to eFGR status.

	Control cohort (n = 32)	**eFGR cohort (n = 29)**	**Significance**
Maternal age (years)*	32.50 ± 4.33	29.36 ± 6.10	P = 0.02
Maternal BMI (kg/m^2)^	25.2 (19.2-35.7)	25.7 (17.6-41.5)	ns (P = 0.54)
Ethnicity†			
White British	28 (87.50%)	20 (71.43%)	
Black	3 (9.38%)	1 (3.57%)	P = 0.02
Asian	0 (0%)	6 (21.43%)	
Mixed	1 (3.12%)	1 (3.57%)	
Parity‡			
Primiparous	13 (40.62%)	13 (46.43%)	ns (P = 0.65)
Multiparous	19 (59.38%)	15 (53.57%)	
Previous pregnancy complications†			
No	6 (31.58%)	4 (26.67%%)	ns (P = 0.53)
Yes	13 (68.42%)	11 (73.33%)	

*Mean ± standard deviation, t-test; ^Median (range), Mann-Whitney U-test; †Counts (percentage), Fisher's exact test; ‡Counts (percentage), Chi-squared test

The pregnancy outcomes differ significantly, as would be expected (Table 5.3). The eFGR cohort gave birth significantly earlier in gestation (30.0 weeks compared to 39.0 weeks; P < 0.001) and the babies had a significantly smaller birthweight and birthweight centile (771g compared to 3232g; P < 0.001, 0th centile compared to 36th centile; P < 0.001) than the control group. Outcomes differed between the two groups, with all pregnancies in the control group ending in survival to discharge, but eight (29%) of the eFGR pregnancies ended in death (four FDIU, four neonatal/infant death). There was no difference between the two groups in terms of distribution of infant sex.

Table 5.3: Pregnancy outcomes for the cohort recruited for FHR monitoring, according to eFGR status.

	Control cohort (n = 32)	**eFGR cohort (n = 29)**	**Significance**
Gestation at delivery (weeks)^	39.0 (36.86-41.86)	30.0 (27.71-33.1)	P < 0.001
Birthweight (g)^	3232 (2460-4466)	771 (420-1426)	P < 0.001
Birthweight centile^	36 (2-90)	0 (0-0.2)	P < 0.001
Infant sex‡			
Male	18 (56.25%)	15 (51.7%)	ns (P = 0.68)
Female	14 (43.75%)	14 (48.3%)	
Pregnancy outcome‡			P = 0.005
Survived to discharge	32 (100%)	20 (71.4%)	

FDIU Neonatal/infant death	0 (0%) 0 (0%)	4 (14.3%) 4 (14.3%)	

^Median (range), Mann-Whitney U-test; ‡Counts (percentage), Chi-squared test

5.3.3. Trace characteristics

Table 5.4 summarises the characteristics of each FHR trace recorded. The number of eFGR recordings reflects the fact that 11 of the eFGR cohort underwent a second recording. Each recording was treated as a separate episode for this portion of the analysis. Those measurements recorded as missing refer to control cases which did not have a scan performed within seven days of the monitoring episode.

Table 5.4: Recording characteristics for each episode of FHR monitoring undertaken.

	Control cohort (n = 32)	**eFGR cohort (n = 40)**	**Significance**
Gestation at recording (weeks)*	28.22 ± 2.3	28.24 ± 2.17	ns (P = 0.97)
Gestation at closest scan (weeks)* Missing	27.97 ± 2.48 7	27.92 ± 2.14 0	ns (P = 0.92)
EFW at closest scan (g) ^ Missing	1227 (531-2133) 7	667 (275-1332) 0	P < 0.001
EFW centile at closest scan^ Missing	67.4 (19.2-100) 7	0 (0-4) 0	P < 0.001
UA Doppler status at closest scan† Normal PI > 95th centile Absent EDF Reversed EDF	 32 (100%) 0 (0%) 0 (0%) 0 (0%)	 2 (5.00%) 9 (22.5%) 23 (57.5%) 6 (15.0%)	 P < 0.001
% signal loss*	38.39 ± 23.77	51.20 ± 29.55	ns (P = 0.06)
Number of hours of useable data Total* Day^ Night^	 13.84 ± 6.08 6 (0-13) 9 (0-10)	 9.71 ± 6.73 3 (0-13) 6 (0-10)	 P = 0.009 ns (P = 0.14) P = 0.007

*Mean ± standard deviation, t-test; ^Median (range), Mann-Whitney U-test; †Counts (percentage), Fisher's exact test

Gestational age was comparable in the two groups, meaning that none of the changes seen between eFGR and non-eFGR cases could be attributed to gestation. Data from the closest recorded scan to the time of the recording (within seven days) were used to look at differences in EFW and UA Doppler status. Seven of the control cohort patients did not have a scan within two weeks of the FHR recording. By design, there was a significant difference in EFW and EFW centile between the control and eFGR cohort, reflecting the extreme nature of the eFGR group. All of the control cohort had a normal UA Doppler, but only two (5.13%) of eFGR cases had a normal UA Doppler, the most common abnormality in this cohort being absent EDF.

Recording quality was compared between the two groups. Although not reaching significance, there was a trend towards a reduced signal loss in the control group (38.39% compared with 51.20% in the eFGR group; P = 0.06). In total, more useable hours of data were captured from each recording in a control participant than from each eFGR recording (13.84 hours compared to 9.71 hours; P = 0.009), which was predominantly due to improved recording overnight (9 hours in the control group compared to 6 hours in the eFGR group; P = 0.007), rather than in the day.

5.3.3.1. *Differences in cCTG criteria between control and eFGR pregnancies*

There were significant differences in cCTG criteria when the FHR in the control group is compared to the eFGR group (Table 5.5). For this portion of the analysis, only the first recorded trace was used for those eFGR cases undergoing more than one recording.

Although there is no difference in the mean FHR, the basal FHR was higher in the eFGR group (141 compared to 137 bpm; P = 0.01), although this difference of 4bpm is unlikely to be of any physiological consequence. eFGR participants had fewer accelerations (both large and small) than the control participants, but there was no difference in the number of large or small decelerations. This may reflect a decrease in HR reactivity or fetal movements in eFGR compared to non-eFGR pregnancies. In terms of variation, both STV and MMR were lower in eFGR, and this is further reflected in the measures of high and low variation, with eFGR cases spending less time in a state of high variation (32.13% of the recording compared to 49.45% of the recording in the control group; P < 0.001), and more time in a state of low variation (5.93% of the recording compared to 0.95% in the control group; P = 0.004). Interestingly the measured MMR during the periods of low variation was lower in the control group, but so little of the recording time in the control group was spent in a state of low variation (less than 1%; 12 control patients spent no time in a state of low variation), that this figure is likely to be unreliable.

Table 5.5: Comparison of cCTG criteria between control and eFGR FHR recordings.

	Control cohort (n = 32)	eFGR cohort (n = 29)	Significance
Mean FHR (bpm)*	139.95 ± 5.14	142.19 ± 4.54	NS (P = 0.09)
Basal FHR (bpm)*	137.31 ± 5.55	141.01 ± 4.71	P < 0.001
Small accelerations (per hour)*	10.72 ± 3.34	7.39 ± 2.38	P < 0.001
Small decelerations (per hour)*	4.08 ± 1.21	3.97 ± 1.15	NS (P = 0.19)
Large accelerations (per hour)†	5.31 (1.30-11.76)	3.00 (1.32-6.00)	P < 0.001
Large decelerations (per hour)†	0.19 (0.00-0.88)	0.17 (0.00-1.33)	P = 0.006
STV (ms)*	10.43 ± 1.49	9.14 ± 1.53	P < 0.001
MMR (ms)*	56.15 ± 7.58	48.87 ± 7.53	P < 0.001
High variation (%)*	49.45 ± 9.90	32.13 ± 14.42	P < 0.001
High variation (ms)*	58.08 ± 6.50	52.00 ± 8.77	P < 0.001
Low variation (%)†	0.95 (0.00- 11.81)	5.93 (0.00-34.09)	P < 0.001
Low variation (ms)†	4.21 (1.10-12.25)	9.53 (2.29-20.54)	P < 0.001

*Mean ± standard deviation, t-test; †median (range), Mann-Whitney U-test

5.3.3.2. *Differences according to umbilical artery flow in eFGR traces*

eFGR traces were further classified according to UA flow at the scan closest to the time of recording. The mean / median of the overall means for each trace were compared. All traces were compared as individual traces for this portion of the analysis as although one patient may have undergone more than one recording, the UA Doppler status may have been different at each recording. Results are summarised in Table 5.6. The only differences between the two groups were seen in the basal FHR, which is 4bpm lower when UA flow is absent or reversed (138.23 ± 6.23 compared to 142.57 ± 4.97 when UA flow is normal or there is raised resistance), and the number of small decelerations per hour, which is highest when UA flow is absent / reversed (4.03 ± 1.38 compared to 2.97 ± 0.84 when UA flow is normal / raised resistance).

Table 5.6: Comparison of standard cCTG parameters in eFGR cases, according to UA Doppler status at scan closest to time of recording. For the purposes of this analysis, UA normal and UA PI > 95th centile are pooled for comparison with UA absent or reversed.

	UA flow present (n = 11)	UA flow absent / reversed (n = 29)	Significance
Mean FHR (bpm)*	143.48 ± 4.69	139.67 ± 5.80	NS (P = 0.06)
Basal FHR (bpm)*	142.57 ± 4.97	138.23 ± 6.23	P = 0.05
Small accelerations (per hour)*	6.37 ± 2.26	7.50 ± 2.40	NS (P = 0.19)
Small decelerations (per hour)*	2.97 ± 0.84	4.03 ± 1.38	P = 0.02
Large accelerations (per hour)†	2.41 (1.32-4.86)	3.64 (1.83-6)	NS (P = 0.12)
Large decelerations (per hour)†	0.06 (0-0.86)	0.22 (0-1.5)	NS (P = 0.26)
STV (ms)*	8.68 ± 1.76	9.38 ± 1.54	NS (P = 0.23)
MMR (ms)*	45.5 ± 8.25	50.49 ± 7.23	NS (P = 0.08)
High variation (%)*	29.77 ± 13.76	32.62 ± 15.52	NS (P = 0.60)
High variation (ms)*	48.37 ± 8.94	54.51 ± 10.40	NS (P = 0.09)
Low variation (%)†	9.85 (0-34.09)	6.22 (0-30.1)	NS (P = 0.25)
Low variation (ms)†	11.37 (6.13-20.54)	12.71 (2.31-28.30)	NS (P = 0.93)

*Mean ± standard deviation, t-test; †median (range), Mann-Whitney U-test

5.3.3.3. Diurnal variation in cCTG parameters

Analysis of the difference between the day and the night was undertaken using two different methods. Firstly, average cCTG parameters for the day and night periods were compared within the control and eFGR cohorts, and secondly mixed level regression was used as a more sensitive method of looking at the relationship between the time of day and the cCTG parameters, including eFGR status as an interaction term.

5.3.3.1. Comparison between day time and night time averaged cCTG parameters

These data are summarised in Table 5.7. Using a straightforward comparison of averaged day and night time parameters shows that in the control group, there was a significant difference in all parameters except for small accelerations and large decelerations. Values were lower in the night time compared to the day time, except for low variation which was higher overnight, suggesting that fetuses spent a longer proportion of time overnight in a state of low variation compared to during the day. This confirms findings from a previous study which investigated the impact of maternal position on fECG overnight in late gestation normal pregnancies (246). In contrast to this, the only cCTG parameters showing a significant day-night variation in the eFGR group were large accelerations (fewer overnight: 2.00/hour compared to 3.00/hour; P < 0.001) and the percentage of time spent in low variation (higher overnight than during the day: 8.30% compared to 0.00%; P = 0.003). The STV showed a trend towards lower values at night time (8.39ms ± 1.64 compared to 9.28ms ± 1.66 during the day), but this did not reach significance (P = 0.07).

Table 5.7: Comparison of cCTG criteria in the day (0700-2259) compared to at night (2300-0659) for the control and eFGR cohorts.

Parameter	Control (n = 32)			eFGR (n = 29)		
	Day	Night	Significance	Day	Night	Significance
Mean FHR (bpm)*	141.99 ± 5.76	138.03 ± 5.47	P = 0.01	143.30 ± 4.55	141.16 ± 5.47	NS (P = 0.15)
Basal FHR (bpm)*	139.10 ± 6.30	135.75 ± 5.91	P = 0.04	142.07 ± 4.85	140.47 ± 5.31	NS (P = 0.29)
Small accelerations/hour*	10.89 ± 3.23	10.00 ± 4.33	NS (P = 0.39)	7.15 ± 3.02	6.57 ± 3.09	NS (P = 0.52))
Small decelerations/hour*	4.79 ± 1.37	3.35 ± 1.46	P < 0.001	4.07 ± 1.29	3.53 ± 1.47	NS (P = 0.19
Large accelerations/hour^	6.00 (0.00-19.00)	4.00 (0.00-19.00)	P < 0.001	3.00 (0.00-16.00)	2.00 (0.00-12.00)	P = 0.001
Large decelerations/hour^	0.00 (0.00-4.00)	0.00 (0.00-3.00)	NS (P = 0.19)	0.00 (0.00-6.00)	0.00 (0.00-4.00)	NS (P = 0.78)
STV (ms)*	11.12 ± 1.55	9.76 ± 1.81	P = 0.003	9.28 ± 1.66	8.39 ± 1.64	NS (P = 0.07)
MMR (ms)*	58.63 ± 7.65	53.53 ± 9.47	P = 0.03	48.73 ± 7.81	45.77 ± 9.15	NS (P = 0.23)
High variation (%)*	41.72 ± 13.00	53.25 ± 14.69	P = 0.003	31.12 ± 14.91	33.52 ± 17.22	NS (P = 0.61)
High variation (ms)*	60.14 ± 8.29	56.31 ± 8.02	NS (P = 0.08)	54.83 ± 9.42	50.70 ± 9.48	NS (P = 0.14)
Low variation (%)^	0.00 (0.00-23.30)	0.00 (0.00-51.70)	P < 0.001	0.00 (0.00-85.00)	8.30 (0.00-76.7)	P = 0.003
Low variation (ms)^	23.40 (16.30-30.40)	24.50 (19.20-36.10)	NS (P = 0.47)	21.90 (13.70-32.50)	22.9 (13.20-33.20)	NS (P = 0.45)

*Mean ± standard deviation, t-test; ^Median (range), Mann-Whitney U-test

5.3.3.2. Longitudinal change throughout the day

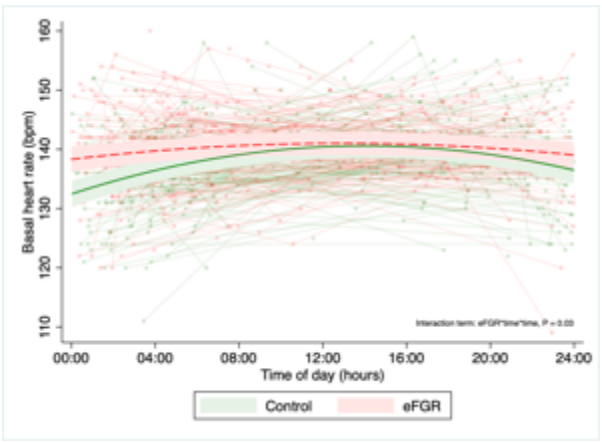
All cCTG parameters showed diurnal variation, and the response throughout the day differed between control and eFGR groups.

The basal FHR showed the most striking difference. In control cases, this was at its lowest at midnight (approximately 132bpm) and rose steadily to reach a plateau at approximately midday (140bpm), then fell again steadily from 1800 to midnight. Conversely, the basal HR follows a significantly different pattern in eFGR (as confirmed by the significant interaction between eFGR status and time of day; P = 0.003) and does not display as pronounced a change throughout (Figure 5.5 (a)). The mean FHR also shows a similar diurnal change, with an increase from midnight to a maximum at around 1600 then a fall to midnight (Figure 5.5 (b)). Although mean FHR appeared to be higher throughout in the eFGR cohort, this is not significant (P = 0.44), and the pattern of response is the same in both groups (no significant interaction between eFGR status and time; P = 0.55).

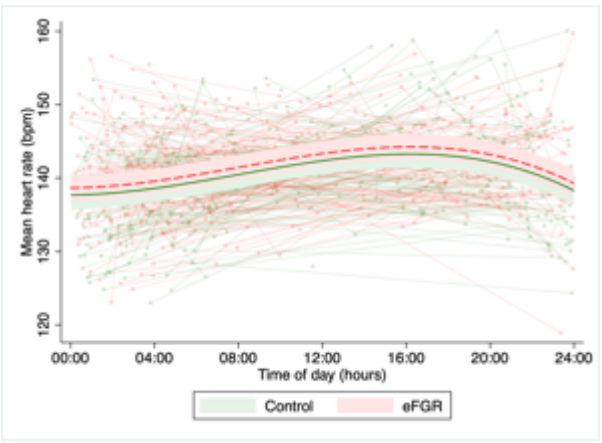
eFGR cases had significantly fewer accelerations (both small (Figure 5.5 (c)) and large (Figure 5.5 (e)) regardless of the time of day (P < 0.001), but the change in pattern throughout the day was the same regardless of eFGR status. The number of accelerations seemed to be highest during the evening (approximately 1900), and lowest in the early morning (approximately 0500), which mirrors the changing pattern of fetal activity (247). This diurnal pattern was also seen with the small decelerations, in both eFGR and control cases (Figure 5.5(d)), but unlike with accelerations, there was no significant difference in the number of small decelerations seen throughout the 24-hour period between the eFGR and control cases (P = 0.24).

Both STV and MMR were significantly influenced by the time of day and eFGR status; as a consequence, periods of high and low variation were also significantly altered (Figure 5.5(f-i)). Both STV and MMR were significantly lower in eFGR than in control cases (P < 0.001 in both; STV 2ms lower; MMR 10ms lower), but the response across 24 hours was the same regardless of eFGR status (non-significant interaction term). Across the whole cohort, both STV and MMR were lowest at 0500, and highest at 1900, which is similar to the pattern seen with the accelerations. The amount of time spent in a state of high variation changes significantly according to eFGR status. In the control group, the highest percentage of time spent in a state of high variation was overnight, with the lowest amount of time around midday. However, the eFGR cases showed a different pattern, and had a lower level of high variation throughout the day compared to the controls, which remained relatively static, and on average 16% lower than the controls (interaction between eFGR status and time of day P < 0.001). In terms of low variation, the diurnal change was the same regardless of eFGR status and mirrors that of the STV and MMR, but the eFGR cases spent 9% more time in a state of low variation throughout the day compared to the control cases.

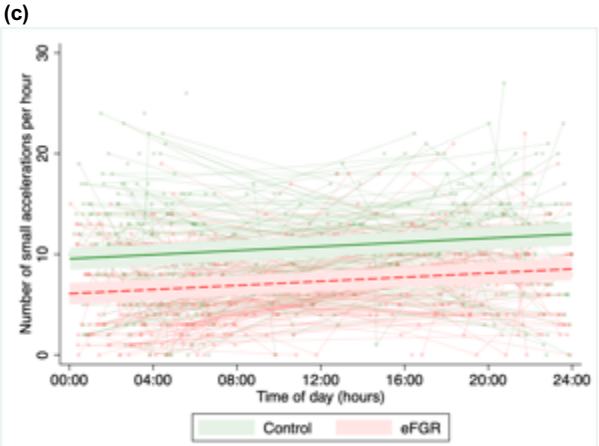
(a)


Basal heart rate (bpm)
110
120
130
140
150
160
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of day (hours)
Control
eFGR

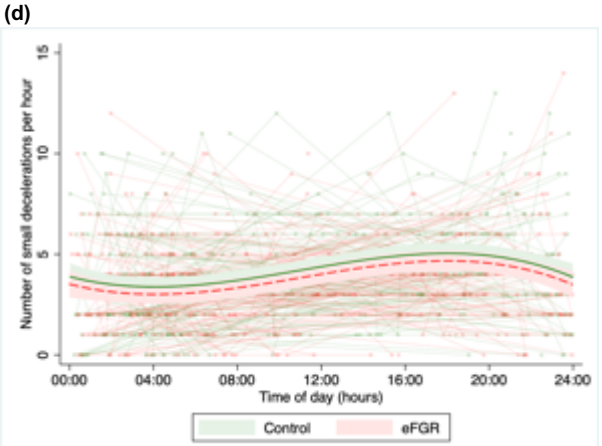
(b)


Mean heart rate (bpm)
120
130
140
150
160
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of day (hours)
Control
eFGR

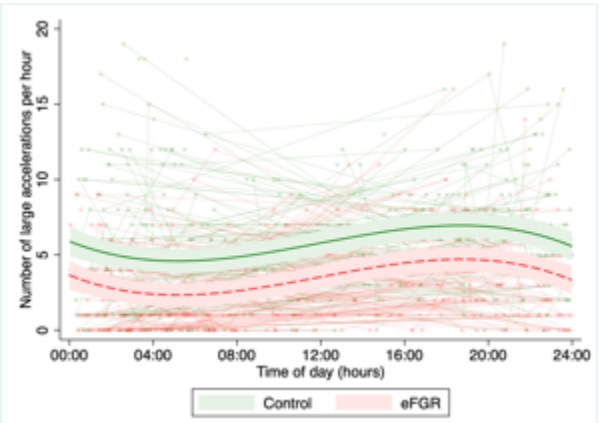
(c)


Number of small accelerations per hour
0
10
20
30
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of day (hours)
Control
eFGR

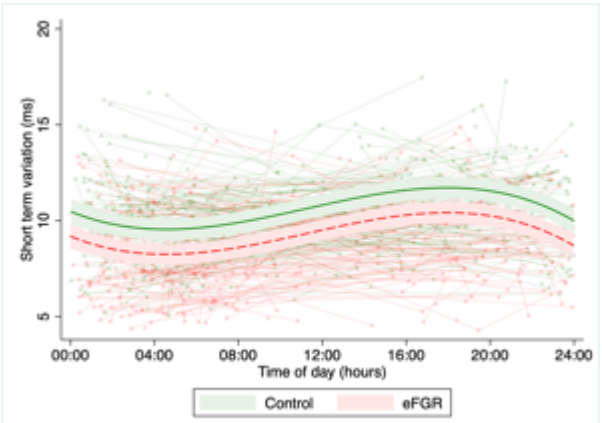
(d)


Number of small decelerations per hour
0
5
10
15
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of day (hours)
Control
eFGR

(e)


Number of large accelerations per hour
0
5
10
15
20
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of day (hours)
Control
eFGR

(f)


Short term variation (ms)
5
10
15
20
00:00
04:00
08:00
12:00
16:00
20:00
24:00
Time of day (hours)
Control
eFGR

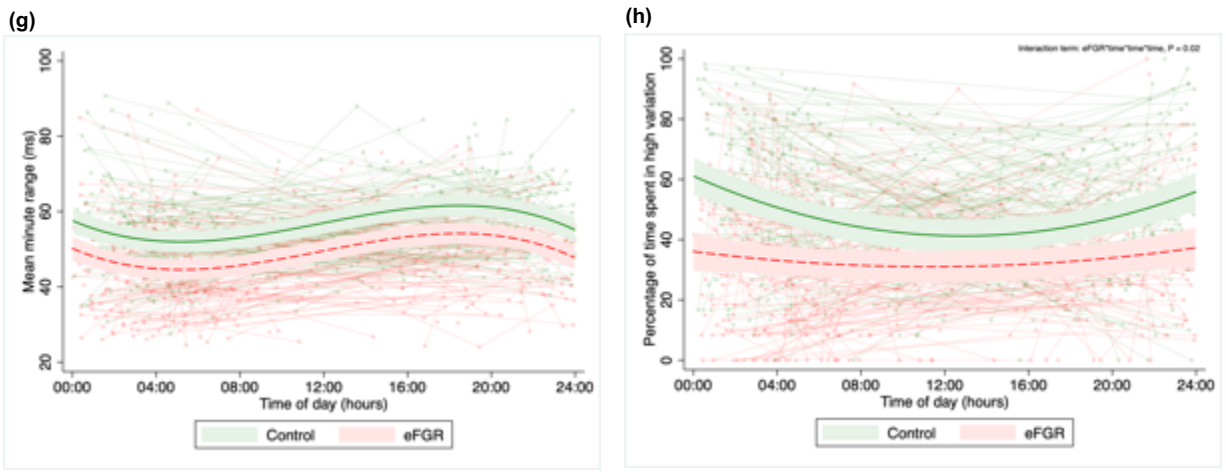


Figure 5.5: Mixed level regression to show longitudinal change of cCTG parameters throughout the day according to eFGR status (control: green; eFGR: red) using a higher order polynomial fitted functions **(a)** Basal FHR; **(b)** Mean FHR; **(c)** Small accelerations/hour; **(d)** Small decelerations/hour; **(e)** Large accelerations/hour; **(f)** STV (ms); **(g)** MMR (ms); **(h)** Percentage of time spent in high variation; **(i)** Percentage of time spent in low variation. Large decelerations and low variation are not shown as no significant relationship with time was found, likely due to the small numbers involved. The average high/low variation during periods of altered variation are not shown as these would not be expected to change with time of day.

5.3.4. Correlation between cCTG parameters

The heat map shown in Figure 5.6 shows how the individual cCTG criteria correlate with one another, and how this differed based on eFGR status. In these heat maps red shows a positive relationship and purple a negative relationship. Figure 5.6(a) shows the heat map for the cohort as a whole, with Figure 5.6(b) showing the correlation between parameters for control pregnancies only and Figure 5.6(c) for eFGR pregnancies only.

The strongest positive correlations were between measures of variation (STV and MMR) and small/large accelerations and small decelerations, and the strongest negative correlations were between the amount of time spent in a low variation state, and the small/large accelerations and small decelerations, and STV/MMR.
When the cohort was analysed according to eFGR status, there are several differences between the two groups:

1. Within eFGR pregnancies, there is a weakly negative correlation between the number of large decelerations per hour and the STV/MMR and amount of time in a high variation state. Within control pregnancies, this relationship is weakly positive.
2. In eFGR, there is a positive relationship between the amount of time in a high variation state and the STV/MMR, but in control pregnancies there is little correlation, potentially due to the significantly higher proportion of time that the control pregnancies spend in a state of high variation.
3. In eFGR, the amount of time in a high variation state correlates negatively with the amount of time in a low variation state, but in control pregnancies there is no relationship, presumably as a reflection of the minimal amount of time that the control pregnancies spend in a state of low variation.

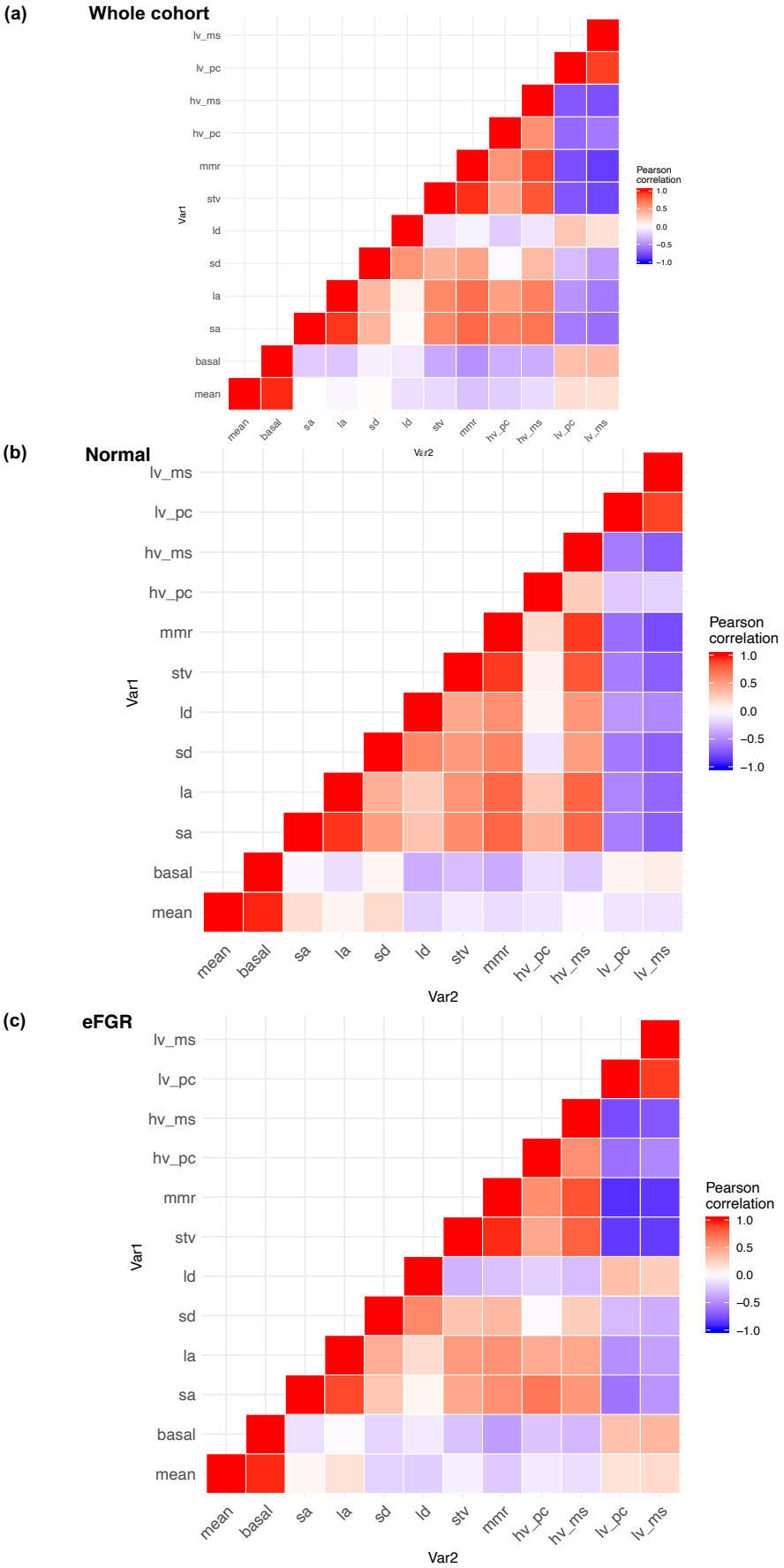


Figure 5.6: Correlation matrix to show relationship between cCTG parameters for **(a)** whole cohort; **(b)** control recordings; **(c)** eFGR recordings. Key: mean: mean FHR; basal: basal FHR; sa: small accelerations; la: large accelerations; sd: small decelerations; ld: large decelerations; stv: short-term variation; mmr: mean minute range; hv_pc: high variation (%); hv_ms: high variation (ms); lv_pc: low variation (%); lv_ms: low variation (ms).

5.3.4.1. *Multivariable analysis*

The final step in this section of the analysis was to determine if combining cCTG parameters could predict eFGR status. Due to collinearity, mean FHR and high / low variation measured in milliseconds were not included in the model. Using a backwards stepwise regression with a P value cut-off of 0.1, eFGR status was best predicted using a combination of MMR, small accelerations and percentage time spent in high variation and in low variation. The model coefficients / odds ratios are summarised in Table 5.8, and Figure 5.7 shows the receiver operating characteristic curve for the model, with a calculated AUC of 0.89 (95% CI 0.82-0.97).

Table 5.8: Results of backwards stepwise regression to determine best combination of cCTG parameters to discriminate eFGR from control pregnancies using cCTG parameters. A cut-off value of 0.1 was used for retention in the final model. Traces were all treated as independent (n = 67 (control traces: 32; eFGR traces: 39)).

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
MMR (ms)	0.14 (–0.02-0.31)	1.15 (0.98-1.36)	0.08
Small accelerations / hour	–0.36 (–0.69- –0.02)	0.70 (0.50-0.97)	0.03
High variation (%)	–0.06 (–0.11- –0.001)	0.94 (0.89-0.99)	0.02
Low variation (%)	0.30 (0.04-0.55)	1.34 (1.04-1.74)	0.05
Constant	–2.99 (–10.9-4.88)	0.05 (0.00-132)	0.46

Pseudo R^2: 0.41

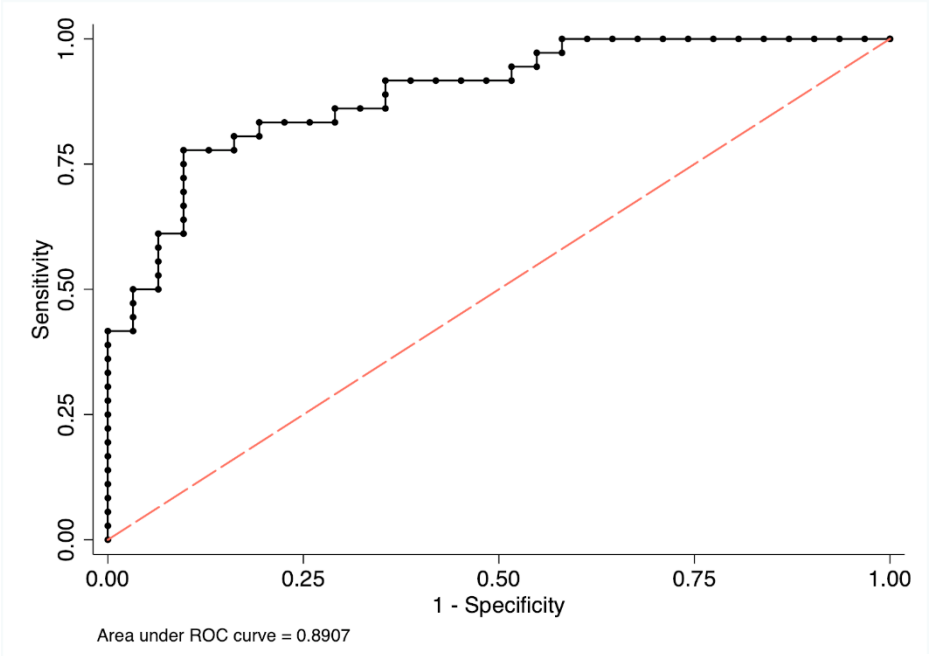


Figure 5.7: ROC curve for model to predict eFGR status using cCTG parameters (MMR, small accelerations, high variation and low variation. AUC = 0.89 (0.82-0.97)).

5.3.5. Non-linear time series analysis

Recurrence analysis techniques were applied to the raw FHR data to produce phase plane plots to facilitate cluster analysis.

5.3.5.1. *Phase plane plots*

An example of the phase plane plots for a control and eFGR case are shown as Figure 5.8.

FHR over time is represented by the x-axis, with the y-axis showing the FHR following the corresponding time lag. The red line at x=y represents the line where points would lie if there was no variation in the FHR during monitoring. As the time lag increases, there is increased scatter of the time points around this line; in the examples shown here the control (black) traces have a more uniform distribution, whereas the eFGR (blue) points are scattered over a greater area and visually appear to be forming discrete clusters.

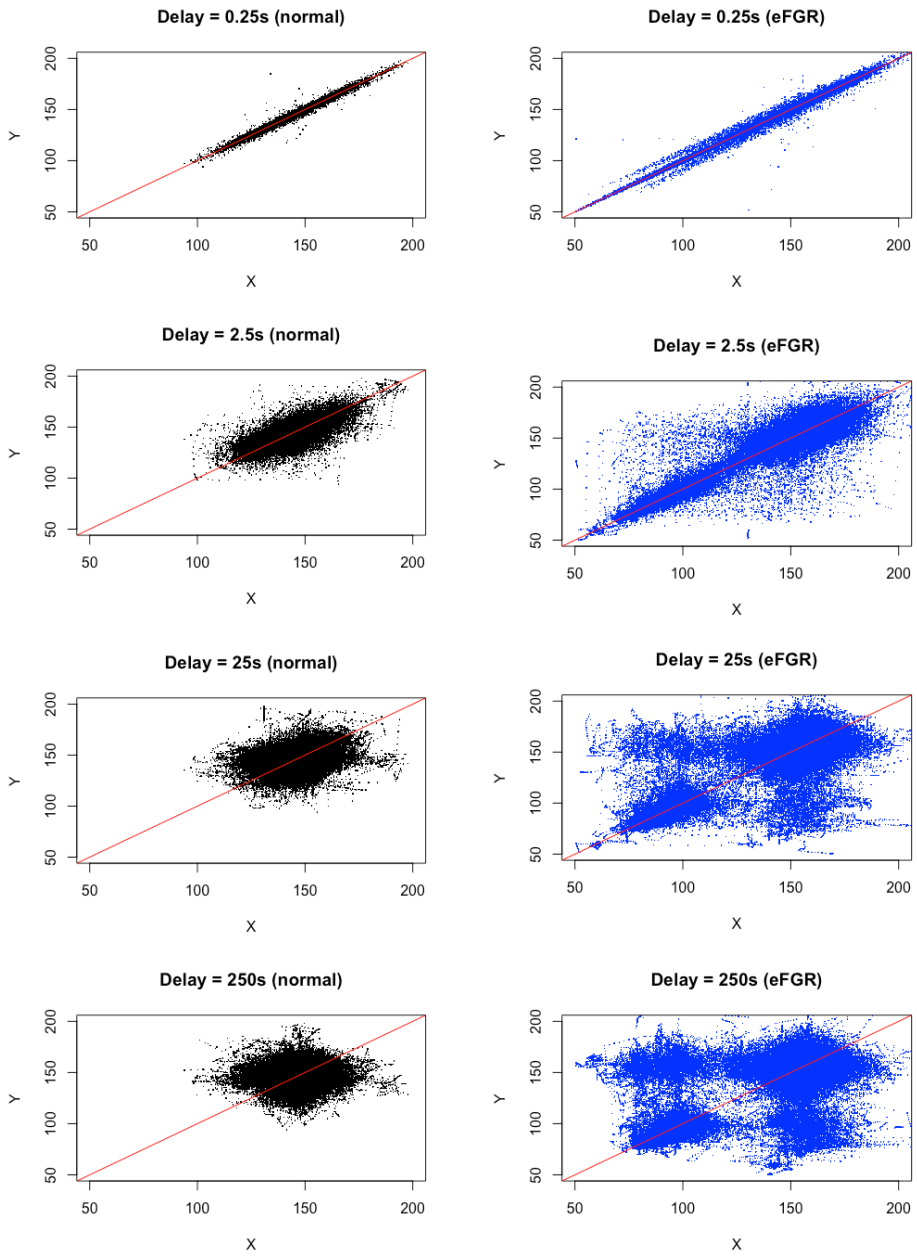


Figure 5.8: Example of phase plane plots at varying time delay intervals for a control (black) trace and an eFGR (blue) trace. As the time lag increases, the clustering of the FHR starts to change in the eFGR traces.

5.3.5.2. *Cluster analysis*

Visual analysis of the phase plane plots shows that distinct clusters are emerging as the time lag increases, and there may be a difference in cluster numbers between control and eFGR pregnancies.

Cluster analysis was performed at time lags of 10, 20, 50, 100, 200 and 500 milliseconds. Figure 5.9 summarises the number of clusters across the whole cohort for each time lag, with time lag plotted on a log scale.

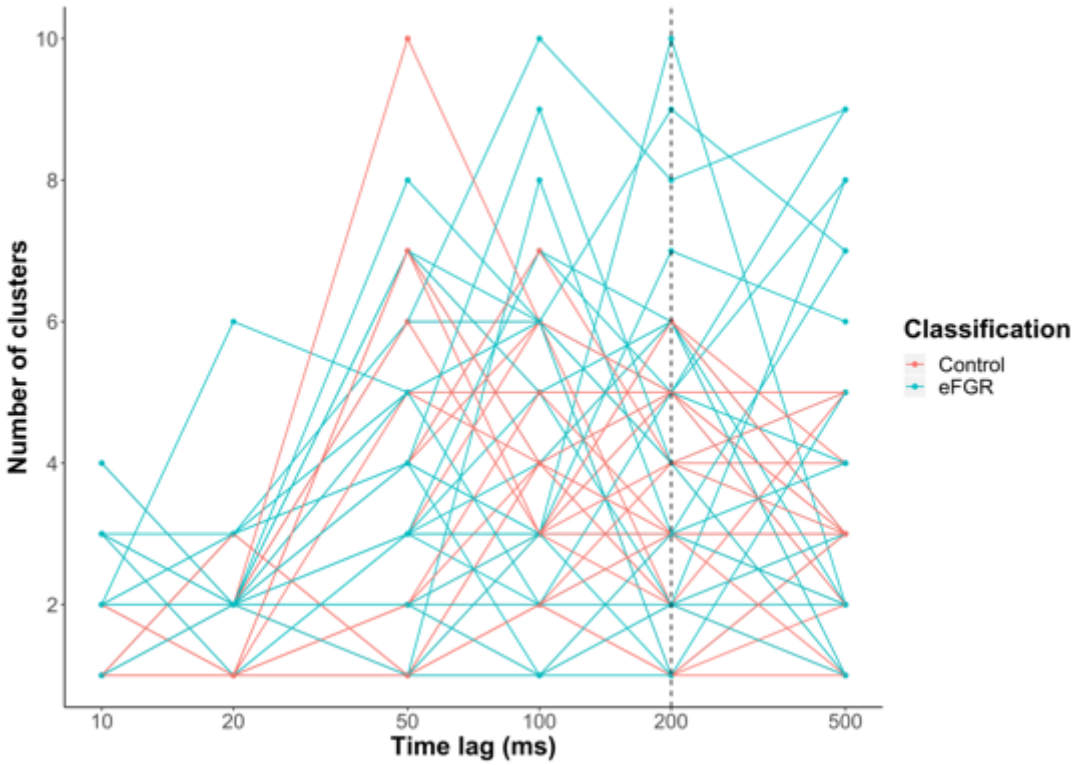


Figure 5.9: Number of clusters formed at each time lag by eFGR status. Each trace is displayed as an individual line. Time lag is displayed using a log scale. Dashed line represents time lag of 200ms.

There does not appear to be a clear relationship between time lag and number of clusters identified, although there is possibly a separation between the two groups above a time lag of 200 milliseconds. As this graph is difficult to interpret, the data are better presented using pie charts, as shown in Figure 5.10, which summarises the cluster results by time lag. For each time lag, a separate pie chart was created for control and eFGR pregnancies to show how many clusters each case displayed at each time point.

Chi-squared analysis was used to investigate if there was a difference in the distribution of clusters between the two groups; results are displayed in Figure 5.10. This showed that at each time lag, there was a difference in cluster distribution. eFGR traces tended to form higher numbers of clusters, particularly as the time lag increased.

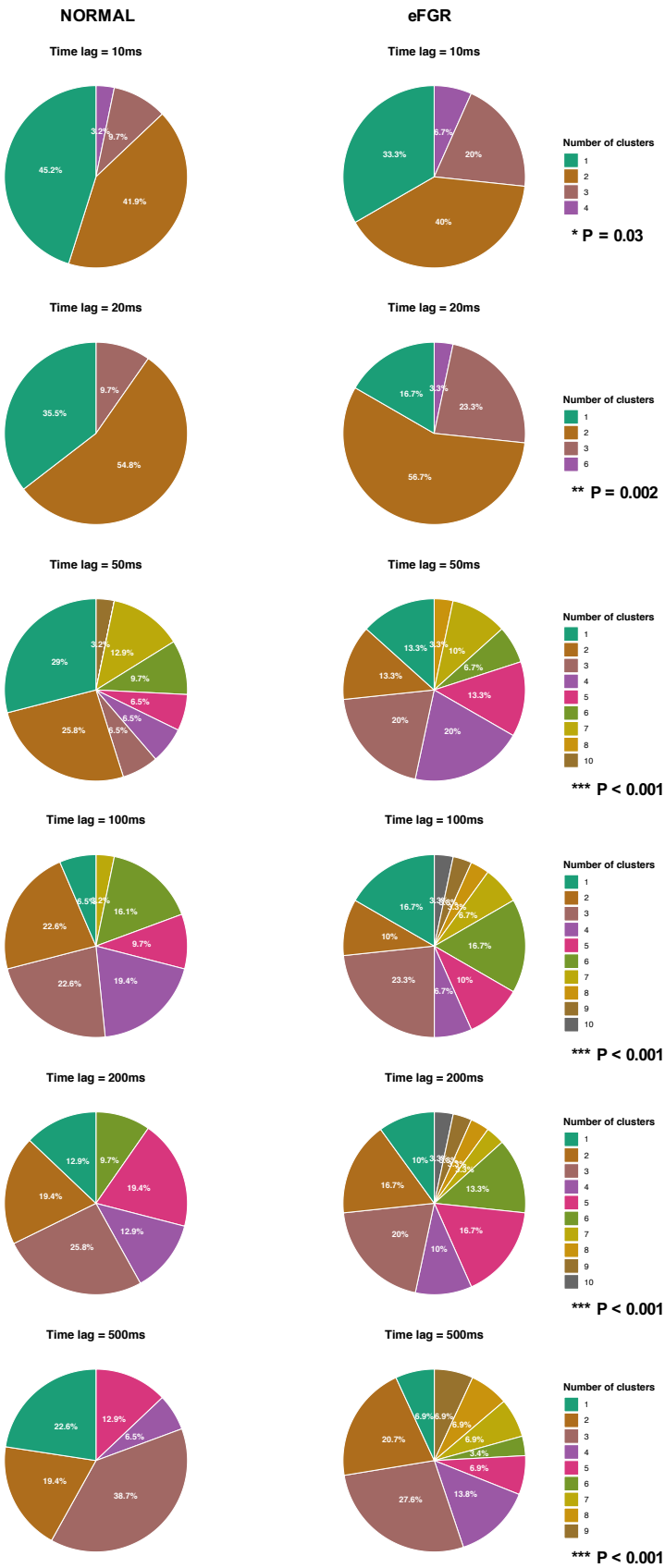


Figure 5.10: The number of clusters formed by each trace at each time lag for control (left hand side) and eFGR (right hand side). For example, at a time lag of 20ms: in the control traces, 35.8% of cases form one cluster, 54.8% form two clusters, 9.7% form three clusters, and 0% form four clusters. This is significantly different to the eFGR traces, where 16.7% form one cluster, 56.7% form two clusters, 23.3% form three clusters and 3.3% form four clusters.

Backwards stepwise regression (using a P value cut-off of 0.1) was used to determine if the cluster number could be incorporated into a model to predict eFGR based on the cCTG trace characteristics. The significant covariates and their coefficients / odds ratios are summarised in Table 5.9 below. The ROC curve for the model is shown in Figure 5.11.

Table 5.9: Results of backwards stepwise regression to determine best combination of cCTG parameters to discriminate eFGR from control pregnancies using cCTG parameters and clusters formed at each time lag. A cut-off value of 0.1 was used for retention in the final model. Traces were all treated as independent (n = 67 (control traces: 32; eFGR traces: 39)).

Covariate	Coefficient (95% confidence interval)	Odds ratio (95% confidence interval)	P value
High variation (%)	–0.08 (–0.15-0.02)	0.92 (0.86-0.98)	0.01
Low variation (%)	0.22 (0.02-0.42)	1.25 (1.02-1.53)	0.03
Number of clusters at 500ms time lag	0.55 (0.07-1.04)	1.74 (1.07-2.82)	0.03
Constant	1.19 (–1.64-4.03)	3.30 (0.19-56.3)	0.41

Pseudo R^2: 0.41

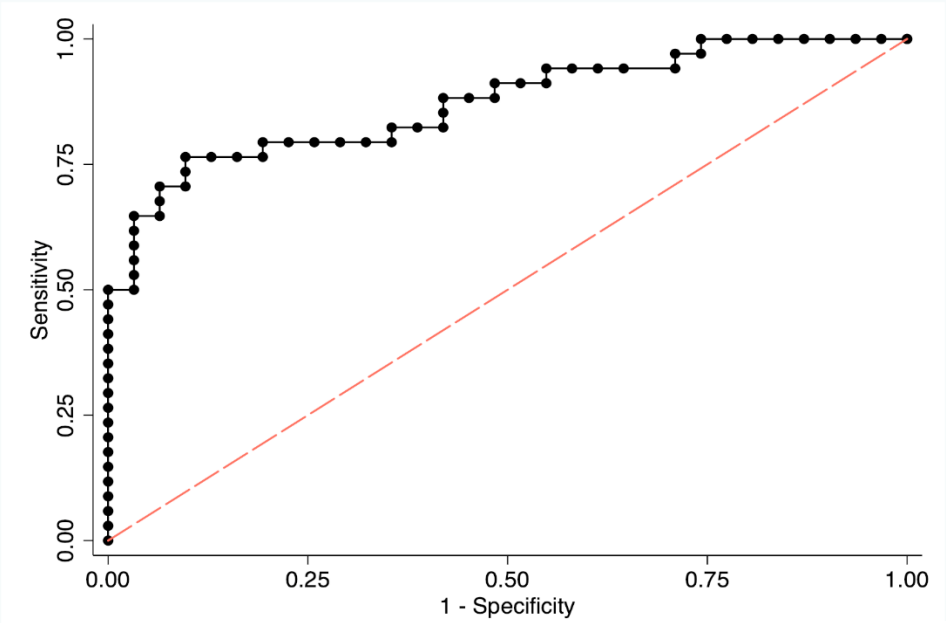


Figure 5.11: ROC curve for model to predict eFGR status using cCTG parameters (high variation and low variation) and results from cluster analysis (number of clusters at time lag of 500ms). AUC = 0.87 (95% CI 0.79-0.96).

This suggests that use of the *k*-means clustering analysis provides an additional method of discriminating between eFGR and control pregnancies. It can be used in combination with cCTG parameters to predict eFGR status, however it does not offer any superior predictive ability over cCTG parameters alone, as shown by the similar AUC value.

5.4. Discussion

This is the first study to compare long-term FHR patterns in low risk and eFGR pregnancies. It highlights that cCTG parameters differ between eFGR pregnancies and healthy controls and there

are differences in changes of these parameters throughout the day. Due to the extended period of fetal monitoring alternative methods of FHR analysis could be explored. These may provide improved discrimination between eFGR and normal pregnancies, and between eFGR pregnancies with normal and abnormal UA Doppler waveforms, and thus could be developed as an additional tool to assess fetal wellbeing and indicate the optimum time of delivery.

5.4.1. Strengths and weaknesses

In this study we used all of the available trace for analysis, whereas previous studies which have used data collected from the Monica AN24 have used randomly selected subsets of the recording (241). The advantage of that approach is that only segments of the trace with acceptable signal quality are included. However, this will introduce bias into the analysis, as sections of the trace with a higher recording quality which are more likely to be selected for analysis may be more likely to be related to periods of lower fetal, maternal or uterine activity, and these variables could exert an influence on the cCTG parameters. Using all of the trace removes this potential for bias but does mean that erroneous readings obtained during periods of low signal quality could be included in any analysis.

Previous studies have reported difficulties recording the fECG between weeks 28 and 34 of gestation due to the negative effect of the vernix caseosa on electrical conduction (24,248). Approximately 50% of the recordings in this analysis occurred after 28 weeks' gestation, and although there was a trend towards increased signal loss post-28 weeks', the difference in signal quality was not significant (before 22.2% $\pm$ 16.1; after 27.0% $\pm$ 16.7; P = 0.24). This suggests that, in our experience, gestation was not significantly detrimental to recording quality, and monitoring the FHR using fECG need not be avoided between 28 and 34 weeks' gestation.

Given that this is the first study to use long-term recording techniques to compare the cCTG in infants with eFGR, an interim power calculation was performed to guide the sample size. This was based on differences that had been noted between eFGR and control pregnancies in terms of large decelerations per hour. This has meant that the study has not been sufficiently powered to discriminate between different pregnancy outcomes in eFGR, which in retrospect would have produced more clinically meaningful results. However, all eFGR pregnancies within the time frame of data collection were recruited to the study, therefore, to capture a larger number of poor pregnancy outcomes, the sample size and data collection period would need to have been much longer than the time available for data collection during the period of this PhD.

5.4.2. Comparison of cCTG criteria

Trace characteristics and recording quality (including the percentage signal loss and the number of hours of useable data) were better in the control group compared to the eFGR group. This is likely related to the increased size of the fetuses undergoing monitoring in the control group, and the reduced presence of oligohydramnios. The heart of an appropriately grown fetus at approximately 20 weeks' gestation is approximately one-tenth the size of an adult heart, with one-fiftieth the voltage of the maternal ECG, therefore there is a low signal-to-noise ratio making filtering the fetal ECG challenging (249). The median EFW at the time of recording in the eFGR cohort was almost

half that of the control cohort, therefore the reduced fetal size could play a role in the reduced fECG recording quality. A fetal echocardiography study has suggested, however, that early-onset FGR is associated with cardiac hypertrophy (250), therefore although the fetus overall is smaller in eFGR, the heart may be proportionately larger which could offset the reduced signal related to the smaller overall fetal size. Nevertheless, despite the increased signal loss associated with the eFGR group, due to the overall length of the recording there was sufficient trace for analysis in the majority of cases from which to draw conclusions.

There were several differences between eFGR and the control group in different domains of the cCTG. Firstly, the basal FHR was significantly higher in eFGR. The mean HR is also higher in eFGR, although this does not reach significance. The mean HR is calculated when the FHR is stable, and excludes accelerations and decelerations, whereas the basal FHR is measured during a period of low variation, and therefore would be expected to be lower (152). eFGR infants spend a significantly higher proportion of the time in a state of low variation, so this may account for the difference seen in basal but not mean HR. Although a highly statistically significant difference exists, the magnitude of change between the two groups was only 4bpm, and both medians are well within the normal limits of FHR measurements (baseline heart rate normal 110-160 bpm (143,147)). One could conclude that this variance, although statistically significant, is not clinically relevant as delivery decisions would never be based on a fluctuation in baseline as small as 4bpm. Physiologically, however, this does confirm a difference between the two groups, which would be worth investigating further as it may reveal more about the pathophysiology or long-term adaptations in eFGR.

Secondly, both small and large accelerations differed between the two groups, with significantly fewer of these events seen in the eFGR group. However, decelerations did not differ between the two groups and in particular large decelerations were relatively uncommon in both groups. This analysis does not, however, take into consideration the length of the deceleration, or the STV/LTV during the decelerative period. A previous longitudinal study in FGR fetuses identified that when LTV started to fall, decelerations started to appear and the mean HR started to increase (159), indicating that the appearance of decelerations is a relatively late stage phenomenon in fetal decompensation. It may be that none of the traces, even in the eFGR group, were performed at a point where an increase in the number of decelerations would be apparent. Accelerations, on the other hand, are believed to be related to a number of processes, including fetal movements (251), which are reduced in times of fetal compensation (252). They are also believed to be under the control of the sympathetic nervous system, and an early study of the FHR in SGA infants suggested that their accelerative capacity after 30 weeks matched the accelerative pattern of an AGA fetus before 30 weeks (253). This potentially indicates a delay in the development of the sympathetic nervous system. Alternatively, the higher basal FHR seen in eFGR fetuses may be secondary to overactivation of the sympathetic nervous system, which could lead to a reduction in the number of accelerations due to its persistent level of high activation.

To summarise, accelerations and decelerations are hypothesised to represent different intrauterine processes. Decelerations are likely a late-stage change, and therefore a significant difference would only be expected between the two groups if one group captured a pre-terminal cohort of infants, which was not the case in this study. Secondly, accelerations are a reassuring feature, and the fact that differences exist between eFGR and healthy fetuses further supports the concept of some sort of difference in fetal adaptation to an adverse *in utero* environment in eFGR. As with the basal FHR data, the magnitude of the difference between the two groups does not have any clinical relevance at present but does indicate there is potential for this difference to provide more information about the altered fetal response in eFGR and how this might change over time.

All measures of variation were significantly altered in eFGR. Both STV and LTV (or MMR) were lower, and the eFGR fetuses overall spent less time in a state of high variation, and more time in a state of low variation than the control groups. The average LTV during the periods of high variation was lower in the eFGR fetuses, but, interestingly, the LTV was higher in the eFGR fetuses during the periods of low variation. This result should be interpreted with caution, however, as overall the control fetuses spent less than 1% in a state of low variation so there will be relatively few data points to analyse.

The autonomic nervous system is responsible for the control of fetal heart rate variability, but the exact nature of the interaction between the sympathetic and parasympathetic nervous systems remains unresolved. Historically, it was believed to be a simple interplay between the two, but experiments performed in fetal lambs suggested it may be more complex than that. Blockade of both sympathetic and parasympathetic drive did not completely abolish heart rate variability as might be expected, but reduced it to about 40%, implying there are likely hormonal and mechanical influences also involved, providing a resting cardio-acceleratory drive (254). Chronotropic hormones, the different stages of fetal sleep and intrinsic variability are also believed to have an influence (255). Recent work using an *ex vivo* sheep model of isolated fetal hearts in conditions designed to mimic chronic hypoxia in the third trimester showed that the fetal heart has an intrinsic control of variability which is affected by chronic hypoxia by negatively impacting short-term FHR variability but increasing long-term variability (256). The measures of FHR variability used in this study suggest that both diastolic function and myocardial contractility are affected, which may affect infant cardiac function in later life (256). Use of fetal magnetocardiography has illustrated that there is a sudden maturation of autonomic function at the end of the second trimester/start of the third trimester which is associated with increasing variability and a shift towards more accelerations than decelerations, which are in turn associated with an increase in parasympathetic modulation (257). This increase in parasympathetic activity, however, is accompanied by a simultaneous increase in sympathetic activation.

The physiological process responsible for the association between low STV and fetal compromise remains unclear. A study using a sheep model of chronic hypoxia in the final trimester of pregnancy showed that hypoxia prevented the usual gestation-related increase in STV and other measures of FHR variability secondary to suppression of the normal sympathetic response (255).

The authors suggest that although this does not explain why sympathetic dysregulation occurs, a reduction in STV could be used as an alternative to a biomarker to highlight pregnancies in which autonomic dysregulation has occurred (255). The main drawback with this conclusion is that although our study has confirmed a lower STV (and other markers of FHR variability) in eFGR, the mean STV still falls well within normal limits ($\geq$ 3.0ms (152)). Although any reduction may well be due to autonomic dysregulation, an isolated measurement which is within normal limits is unlikely to raise suspicion of chronic hypoxia.

Features of the FHR are controlled by a complex interplay of autonomic nervous system interaction. In short, parasympathetic activation is responsible for a decrease in the FHR and sympathetic activation an increase in FHR. Accelerations and increased variability are thought to be modulated by parasympathetic activation. The results from this analysis present conflicting evidence as to whether the changes seen are as a result of an imbalance in the autonomic nervous system or immaturity of the sympathetic nervous system. With nervous system maturation occurring around 30 weeks' gestation, it is feasible that some of the differences seen in eFGR could be related to autonomic immaturity. It has been hypothesised that chronic hypoxia which suppresses the usual sympathetic drive could be responsible for the reduction seen in STV. However, the reduction in accelerations and STV/LTV is also hypothesised to be related to a reduction in parasympathetic drive, or an increase in sympathetic drive, potentially reflecting increased fetal stress secondary to chronic hypoxia *in utero*. It is therefore difficult to conclude if the sympathetic nervous system is overactive due to stress, or underactive due to functional immaturity in eFGR.

The other key message relates to the level of clinical relevance of these measures. As previously discussed, although definite differences are seen between eFGR and controls, the magnitude of these differences is small, and values for both cohorts still fall within normal ranges. This means that it is clinically impossible to distinguish between what would be classed as normal variation in measurements and variation that is related to physiological differences.

5.4.3. Diurnal variation

The other aspect to these results relates to the circadian rhythm associated with the cCTG parameters. The concept of a circadian pattern in the FHR as early as 20-22 weeks' gestation is well established (258), and although in adults the circadian variation is driven by the autonomic regulation of the suprachiasmatic nucleus in the hypothalamus (259), the origin of fetal circadian variation remains unknown. A previous study has investigated fetal circadian patterns using the Monica AN24 throughout the day/night in 54 women with uncomplicated pregnancies between 25 and 40 weeks' of pregnancy. The main findings included a night-time reduction in the basal HR and an increase in STV/LTV and accelerations (260). Our findings that the basal FHR is lowest at 0000hrs and highest at 1200hrs corroborate those from Kapaya et al. (260), but the finding that STV/LTV and accelerations are lowest at 0500hrs and highest at 1900hrs are not in keeping with their findings. This difference could be related to the different methods of analysis used. Kapaya et al. randomly selected three 30 minute frames from the day to compare with three from the night for

each subject (260), whereas in this study all data obtained from the recording have been used in a mixed level regression to account for different time points and different individuals within each group. The advantage of the approach employed here is that it provides a longitudinal pattern of change which can be visualised over a 24 hour period and may reveal more subtle changes, rather than just using average point values for comparison.

A subsequent study by Kapaya et al. (241) compared differences in diurnal variation between AGA and SGA fetuses. The SGA group investigated in that study was much less extreme than our eFGR cohort, with the mean gestation at delivery being 39.0 weeks and the mean birthweight 2930g, but the diurnal changes seen in AGA infants were not mirrored in SGA infants, with the only parameter showing a significant difference between the day and night in SGA being the basal FHR (241). Our data corroborate these findings, with significant differences in parameters between the day and night seen in the control group, but not in the eFGR group.

Reassuringly, the pattern in diurnal variation seen in the control pregnancies reflects knowledge regarding maternal perception of fetal movements in the third trimester in healthy pregnancies. Fetal movements have been reported to be strongest in the evening (247), which coincides with when this study reports the highest variability in the FHR, with STV, LTV, accelerations and decelerations, all of which are related to fetal movements (251), all being highest in the evening.

Clinically, if a natural diurnal variation exists in measures of the cCTG then this could have implications regarding when the cCTG should be measured or at least that the cCTG should be assessed at a consistent point in time. During periods of the day when the STV is lowest (from this study this would be overnight), clinicians should potentially refrain from using cCTG monitoring. Particularly in cases of eFGR, where the STV has been shown to be lower regardless of the time of the day, it should be monitored during a period when the time of day will not be a contributing factor. Having said that, there is no evidence that the magnitude of change throughout the day should be enough to account for an STV within the range that suggests acute hypoxia, and this should still be acted on in the appropriate manner.

In terms of prognosis, further research needs to be undertaken to determine if the magnitude of difference between control and eFGR relates to pregnancy outcome. In addition, splitting the eFGR cohort according to pregnancy outcome and reanalysing the diurnal patterns may reveal differences. For example, it could be hypothesised that in the most severe cases that are more likely to result in death, there could be a significant reduction in FHR variability. Alternatively, the increased levels of stress could result in even higher than normal sympathetic nervous system activation which could cause a lack of the usual variability and accelerations. If differences are found to exist, then longitudinal monitoring of the STV could be used as an adjunct to ultrasound scanning and potentially as an extra predictive factor to better inform of the likely outcome in eFGR.

5.4.4. Prediction of eFGR status

It is possible to predict eFGR status using a combination of cCTG parameters (MMR, small accelerations, high and low variation). The calculated AUC 0.89 (0.82-0.97) is actually higher than that of the ultrasound-based predictive model to predict FDIU presented in Chapter 3 (0.82 (0.74-0.91)). However, at the point of performing a cCTG the diagnosis of eFGR is known, and cCTG would never be used as a tool for diagnosis above ultrasound, so this has little clinical utility.

5.4.5. Recurrence analysis

Using recurrence analysis is a novel method of interrogating the FHR. This study has shown that differential patterns exist between control and eFGR pregnancies in terms of the number of clusters present at different time lags. At each time lag, the number of clusters that the FHR split into was higher in the eFGR recordings than the control recordings. The higher the number of clusters that the phase plane plot is split into implies that the FHR covers a greater spread of values, and therefore is more variable. However, analysis of the cCTG shows that this is not the case, with both STV and measures of LTV reduced in eFGR cases. Use of recurrence analysis techniques to investigate the response of adult heart rate variability to mental stress has suggested that stress leads to increased clustering of points in the phase plane plot (261), as is seen here in the presence of physical stress, confirming cluster analysis as a potential method of investigating stress. The finding that cluster analysis differed between control and eFGR pregnancies, with eFGR pregnancies forming a greater number of clusters as the time lag increases, led to inclusion of these data in the multivariable analysis. The number of clusters formed at 500ms was found to be predictive of eFGR status when combined with high and low variation measures. This did not add any predictive value, however, over the model which just used conventional cCTG parameters, suggesting that the apparent differences in cluster analysis are no more predictive than cCTG parameters alone. Furthermore, calculation of the number of clusters is time consuming and impractical from a clinical perspective.

5.4.6. Future work

The findings presented in this chapter confirm that longer-term monitoring using cCTG does highlight differences in eFGR pregnancies. This suggests that there are important physiological differences in the control of the FHR in eFGR which are worth investigating further to expand understanding of the pathophysiology of eFGR.

It has been speculated that a STV < 3ms is a pre-terminal change in the cCTG. To investigate this further, FHR monitoring would have to be undertaken on fetuses near the point of demise, which has obvious ethical implications. Using the study protocol outlined in this study could capture this type of data. Patients with an eFGR fetus who underwent monitoring in this study were always made aware of the potential for fetal demise during the monitoring period, due to the severe nature of eFGR.

PRSA has been previously suggested as a method of investigating FHR variability, and has shown ability to predict outcome in cases of early-onset FGR earlier than STV, through a change in AAC

and ADC (171). Such analysis techniques could be applied to the dataset collected in this study to determine if there is a change in the AAC or ADC in eFGR pregnancies approaching the time of delivery or if it can be used to discriminate between severity on the basis of UA Doppler status. Future work could involve a second prospective study to perform multiple recordings in eFGR pregnancies to gather longitudinal data on the AAC and ADC and relate this to pregnancy outcome. Lobmaier *et al.* identified that the change in AAC between five days and 24 hours prior to delivery was predictive of FDIU, however they were limited to five days due to the TRUFFLE data collection (171). Performing a longitudinal study at prespecified time points from the point of diagnosis to delivery could allow an earlier change in PRSA parameters that is predictive of outcome to be identified. This would add to the predictive capacity offered by the model discussed in Chapter 3.

5.4.7 Conclusion

Although we have identified a difference in cCTG parameters between the eFGR and control pregnancies, in its present form this does not add any clinical benefit to those tools already available. At the time of cCTG monitoring, eFGR status is likely already known. Instead of being able to discriminate between eFGR and non-eFGR pregnancies, which can already be accurately done using ultrasonography, it would be more useful to be able to use the novel information derived from cCTG to identify those fetuses approaching decompensation and who require delivery to reduce the chances of perinatal death. Due to the huge benefits from administration of antenatal steroid and magnesium sulphate at this gestation, any delivery indicator ideally needs to account for the 24-48 hours that are required to facilitate this. Notably, none of the traces collected in this study ever had an STV recording as low as that which was recommended as a trigger for delivery in the TRUFFLE study (<3.0ms) (76), despite several recordings taking place in severely growth restricted fetuses immediately prior to delivery. This highlights the fact that an STV < 3.0ms must be a pre-terminal event, and a better metric is likely required for antenatal surveillance. The fact that this study has identified a difference between eFGR and control pregnancies shows that some distinction exists in heart rate variability in eFGR, regardless of gestation or apparent level of compensation / decompensation to adverse *in utero* environment. Accordingly, it is hoped that such observations could be extrapolated to identify those at a higher risk of fetal demise. However, the only way to capture potential changes in the period immediately prior to death would be to monitor fetuses to the point of death. This could only feasibly and ethically be done in those cases where delivery was indicated for maternal reasons at extremes of birthweight and gestation incompatible with life. It would therefore be difficult to say whether any changes noted could be attributed to death, rather than gestation or birthweight, or related to the maternal disease. On the other hand, it seems that STV < 3.0ms represents these pre-terminal changes, therefore future work could focus on the period before this pre-terminal state, when although delivery is indicated, there is time to plan for it.

The results from this study are consistent with previous work which has examined the cCTG in FGR pregnancies, but not specifically to eFGR. Alternative methods of FHR analysis have been proposed as methods of better discrimination between normal and eFGR pregnancies. Although

this work has been useful in identifying differences, from a clinical perspective further work is required in order to link differences to prognosis and clinical outcome. Identifying differences is useful from a physiological point of view and improves our understanding about the pathophysiology of eFGR, but the aim of this thesis is to improve the management of eFGR. Therefore, further work needs to concentrate on how the differences identified in cCTG can be taken forwards to identify a method of analysing the FHR that can be used to predict prognosis in eFGR.

CHAPTER 6: DISCUSSION

The work presented in this thesis has advanced the understanding of eFGR in terms of incidence and survival estimates on a population level and determined factors predictive of outcome on an individual level. Previous research in FGR has predominantly split FGR into an early- and a late-stage disease, differentiated by a gestational age of 32-34 weeks (262). However, following the TRUFFLE (76) and STRIDER (15) studies, which focused on early-onset FGR, it became apparent that within pregnancies diagnosed as having early-onset FGR there was a more severely affected subtype with high associated rates of mortality and morbidity. The majority of babies with early-onset FGR are delivered after 32-34 weeks' gestation. With modern neonatal care, survival in this group is high, with overall intact survival estimated at 96.8% for infants born between 32-34 weeks' gestation (65). Within the extremely early FGR subtype, delivery at 32 weeks' is often the maximum gestation attained due to fetal Doppler changes (231). Therefore, this thesis aimed to focus specifically on this extreme cohort due to its poor prognosis and disproportionate contribution to perinatal mortality.

Using the population level data as described in Chapter 2, this study has defined the incidence of eFGR to be 3 per 1000 births in the general maternity population. This confirms previous estimates which used the incidence of pre-term pre-eclampsia as a proxy measure. Using a smaller local data cohort collected from a single tertiary-level unit has suggested that the incidence in a specialised unit is 5 per 1000 births. Confirming disease incidence is crucial for many aspects of patient care, not least to determine the workload and the economic cost. Combining this knowledge with the findings from Chapter 4 will allow a tertiary-level unit to determine the economic impact that eFGR pregnancies have on antenatal, intrapartum and postnatal care for the mother and neonatal care for surviving babies. In a large tertiary unit with 7500 births per year, these data suggest that there would be up to 38 cases of eFGR annually (including referrals from other regions), or approximately three per month. The responsibility of care for these pregnancies would normally fall within the remit of the Fetal Medicine Unit, suggesting that on average 30 scans per month (assuming an average of 10 per patient) would be required to facilitate the intensive antenatal surveillance associated with eFGR. In addition, from the perspective of a small district general hospital with 4000 births per annum for example, this information confirms that they could expect to see 12 eFGR cases per year, or one per month. Although the majority of the care following a diagnosis of eFGR would take place in a specialist unit, some scans may still be performed locally, and this information would help a smaller hospital determine if they can accommodate the additional scan capacity required.

The work presented in Chapters 2 and 3 has confirmed the high associated rates of mortality and morbidity in eFGR. Estimates of FDIU within this cohort range from 21% (NHS Scotland population data; Chapter 2) to 27% (St Mary's local cohort data; Chapter 2) and estimates of neonatal death range from 6.2% (NHS Scotland population data; Chapter 2) to 14% (detailed St Mary's retrospective cohort; Chapter 3). In the UK in 2018, the overall FDIU rate was 3.5 per 1000 (0.35%) and the neonatal death rate was 1.6 per 1000 (0.16%) (263). 54% of FDIUs and 56% of neonatal

deaths occur in babies born before 32 weeks' gestation (263). Despite eFGR being uncommon, a reduction in the associated rates of FDIU and neonatal death in this cohort would therefore translate into a large impact on overall death rates.

The conclusions drawn from this thesis will influence various different aspects of eFGR: namely clinical care and clinician behaviour, health economics and resource allocation, and the counselling of affected families.

6.1. Clinical perspective

From the point of view of clinical care, the findings of this thesis are relevant to both obstetricians and neonatologists. The remit of this thesis was not to find a treatment or determine the most appropriate delivery indication, but to use the tools and knowledge already available to be able to offer prognostic information. The use of population level data in Chapter 2 to understand the relationship between gestation at delivery, birthweight and pregnancy outcome has confirmed that both are important in eFGR but has failed to provide a clinically meaningful prediction model. Increasing gestation and increasing birthweight both confer a survival advantage regardless of eFGR status; however, being eFGR reduces the likelihood of survival at a given gestation but increases the likelihood of survival at a given birthweight because affected fetuses reach that birthweight at a later gestation than their appropriately grown counterparts. Combining the two factors to predict outcome suggests that increasing gestation in the absence of increasing weight does not increase the probability of survival. Static growth can be a feature of eFGR, therefore this would suggest that in the absence of weight gain, gaining gestation is not of immediate survival benefit. This does not take into account the risk of FDIU, and this work was not able to determine how the risk of FDIU changes as the pregnancy advances, particularly in the context of static growth. These data and the conclusions drawn from them are, however, heavily influenced by clinician behaviour. The majority of eFGR births will be iatrogenic, therefore both the gestational age at delivery and the birthweight are influenced by the decision of the clinician on when to act to deliver. This will create bias in the data with those fetuses at a periviable weight potentially not receiving active management, and not receiving the same level of resuscitation were they to be born alive, and there is no accounting for this in the modelling performed.

Personalised medicine and individual risk prediction models are developing in many domains of medicine. In obstetrics, the QUiPP App has been developed as a method of predicting pre-term birth on an individual level and has reduced unnecessary admissions that result from treatment decisions made on population level data (264). Chapter 3 provides the beginnings of a prognostic factor study which has identified that gestation-adjusted estimated fetal weight, amniotic fluid index, umbilical artery Doppler status and UtA RI are predictive of FDIU at the time of diagnosis. This will require testing in a prospective cohort to determine if it is clinically valid but provides the beginnings of personalised care in eFGR. Chapter 4 confirms that eFGR patients undergo regular growth and Doppler scans and illustrates that this provides an opportunity to continually refine that prognosis prediction if longitudinal data can be incorporated into prediction. Chapter 3 has quantified the relationship between fetal weight gain and FDIU by suggesting that 50g weekly

weight gain between 24^{+0}-25^{+6} and 26^{+0}-27^{+6} weeks' gestation confers a 47% and 55% reduction in the risk of FDIU respectively. This is an example of how longitudinal data can be used as a prognostic factor and highlights the potential wealth of information that can be gained from longitudinal data analysis in eFGR.

An alternative method of longitudinal analysis is explored in Chapter 5, by using FHR monitoring. Computerised CTG already has an established role in the management of eFGR through monitoring of the STV (80). Chapter 5 has confirmed that differences in cCTG parameters exist between normal and eFGR fetuses and that in eFGR there is a lack of the usual diurnal variation. However, these findings are not of a sufficient magnitude to have a direct clinical use, but are suggestive of an altered physiological state within the eFGR fetus, potentially in the control of the autonomic nervous system (254), which warrants further research to elucidate the pathogenic mechanism. An alternative method of CTG analysis which has shown promise here and has been proposed in other work is use of PRSA and the AAC and ADC of the FHR (173,265). Conclusions from Chapter 5 suggest that a change is observed in these parameters approaching delivery, and that this may occur slightly earlier than the change in STV (155). This would therefore give clinicians firstly an alternative measurement to provide prognostic information other than those widely used from ultrasound scans, and secondly a longer time period in which to prepare for delivery. Timely administration of antenatal steroids (to reduce respiratory complications) (227) and magnesium sulphate (for neuroprotection to prevent cerebral palsy) (228) is key to optimise the condition in which the infant is delivered in eFGR and give the neonatology team time to ensure the appropriate availability of care.

6.2. Health economic perspective

Chapter 4 provides a summary of the amount of antenatal care that each eFGR case requires, and an estimate of the neonatal length of stay. This provides a framework which can be used to start to assess the economic impact of eFGR on the health service. One of the conclusions from the data is that over the study period, practice has evolved to prolong the antenatal period and increase the number of surveillance scans performed (increase in Doppler scans), but without a resulting change in the neonatal length of stay. This would suggest that currently more money is being spent in the antenatal period than was spent at the start of the study period, but it does not take into account the level of neonatal care received, and the ongoing care required for these infants following discharge. For example, an association has been suggested between absent EDF in the UA and the presence of NEC (266). If prolonging the interval between absent EDF and delivery (as Chapter 4 suggests has occurred) results in higher rates of gastrointestinal complications in the infant then not only will antenatal costs be higher due to the longer antenatal period, but also neonatal costs will be higher due to the intensity and length of the treatment required. On the other hand, if prolonging the gestation results in a shorter duration of intensive treatment then this would negate the higher costs associated with a prolonged antenatal period. A day's NICU care averages £1531, but high dependency averages £1007 per day (226), therefore a prolonged antenatal period could still reduce overall costs if the resulting neonatal overall length of stay remains the same, but the proportion of time spent in NICU / high dependency care is changed. Improving antenatal care

has been highlighted as a strategy for reducing the costs associated with NICU care (267). St Mary's Hospital has a specialised growth clinic where the majority of the eFGR cases are seen, but in most units eFGR pregnancies would be cared for within the Fetal Medicine Unit (268). The information in this chapter could be used by other tertiary units considering implementing a similar fetal growth clinic.

6.3. Patient perspective

Finally, it is hoped that the findings from this thesis can contribute towards an improved patient experience in eFGR. The overarching goal from improving the management of eFGR is to prevent a family from having to experience either an FDIU or neonatal death. When faced with this scenario, the first question that a patient usually asks is "will my baby survive?" Although the conclusions from Chapters 2 and 3 do not provide a definitive answer to this question, they do contribute to providing prognostic information to better prepare parents for the potential course of the pregnancy that lies ahead. Unfortunately, due to the nature of eFGR some cases will never reach a viable weight, and there are parents that will opt for passive management, or termination of pregnancy. Even for those cases where death is unfortunately inevitable, it is hoped that by improving the prognostic information we can offer to parents we can make small improvements to the patient experience of eFGR.

6.4. Future work

There are several avenues of future work which have arisen from the conclusions in this thesis, to further advance knowledge surrounding the management of eFGR.

6.4.1. Prognostic research

- Expand the population level study to explore the relationship between gestational age at delivery, birthweight and survival in eFGR specifically in tertiary-level units, where care is more likely to be unified to better estimate survival in preterm infants.
- Model the relationship between gestational age, birthweight and survival in eFGR infants using a competing risks model to allow inclusion of cases ending in FDIU.
- Perform a multi-centre prognostic factor study to expand on the existing prognostic factor study presented in this thesis and identify additional factors prognostic of outcome in eFGR.
- Perform a prospective study to test the external validity and, subsequently, clinical impact of the proposed prognostic model in a population of eFGR pregnancies.

6.4.2. Antenatal fetal heart rate monitoring

- Assess the value of PRSA as an additional prognostic indicator in eFGR. This would require serial FHR monitoring to be undertaken up until the point of delivery, to determine if there are changes that occur prior to delivery that identify fetal compromise earlier than the STV. Such data would be collected alongside data regarding fetal growth and maternal / fetal Doppler parameters, and administration of antenatal corticosteroids and magnesium sulphate.

6.4.3. Economic impact

- Further investigate the level of care required for ongoing eFGR management in the neonatal period to provide a more accurate assessment of associated healthcare costs and determine if prolonging the interval between diagnosis and delivery shortens the length of time an infant requires level 3 care.
- Perform a longer-term (two year) follow up study to investigate the morbidity associated with eFGR and assess the longer-term economic impact of eFGR beyond the immediate neonatal period.

6.5. Conclusion

Identification of factors prognostic of eFGR outcome has shown how individualised care can be applied to the management of eFGR. This, coupled with the findings regarding the relationship between gestational age, birthweight and survival, will better inform clinicians regarding pregnancy outcomes, leading to improved counselling of affected parents. Differences in computerised CTG parameters have been identified in eFGR, and although not clinically relevant, they offer new avenues to explore how CTG can be used as an additional prognostic tool, to improve the prediction of pregnancy outcomes in this uncommon, but potentially devastating condition. Combining this knowledge with the proposed future work will translate to improved management of eFGR, and a subsequent reduction in associated morbidity and mortality.

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