Placental Pathology in Twin Pregnancies

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Candidate Thesis Declaration

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

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Summary

Twin pregnancies remain a challenge in perinatal medicine, with significantly higher rates of complications and increased perinatal morbidity and mortality when compared to singletons. Abnormal growth in twins is a significant independent contributory factor to this increased morbidity, and knowledge of the underlying pathological processes governing growth disorders in twins is vital to improving our antenatal surveillance and clinical management of these pregnancies.

We performed a comprehensive study of the placental factors governing growth and development of twin pregnancies in a large, prospectively collected cohort of twins. Placental pathology was evaluated in terms of gross placental abnormalities and histopathological variants with further stereological studies then performed on a selected cohort of placentas. The findings of this study emphasised the differing pathologies underlying disordered growth in monochorionic and dichorionic twin pregnancies. Placentas of monochorionic twins with discordant growth and intrauterine growth restriction showed increased rates of gross abnormalities such as abnormalities of umbilical cord insertion. This likely reflects the unequal placental sharing that may lead to growth discordance among these twins. In contrast, placentas from dichorionic twins with abnormal growth displayed histopathological features consistent with uteroplacental insufficiency.

The stereological studies of dichorionic twins further confirmed this with placentas from growth restricted dichorionic twins displaying a reduction in surface area and volume of terminal villi and capillaries. In addition the stereological studies contrasting normal twins and normal singletons gave an interesting insight into the adaptive mechanisms at play to maintain growth in multiple pregnancies.

The findings of this study reinforce the importance of accurate assessment of chorionicity in twins, targeted ultrasound assessment...
including evaluation of placental cord insertion and selected use of multi-vessel Doppler studies to evaluate fetal well-being as part of antenatal ultrasound surveillance.
For Brian, Miles and Ted
Chapter 1 Introduction

1. 1 Twin Pregnancies

1.1.1 Incidence and Complications

Twin pregnancies and higher order multiple gestations have been increasing in frequency over the past number of decades. The increased rates of perinatal morbidity and mortality observed in twin pregnancies place an increasing demand on maternity services.

Rates of twin pregnancy in Ireland are currently estimated at approximately 35 per 1,000 maternities (1). This compares to a range of 24 to 40 per 1,000 births across European countries (1) and 33.7 per 1,000 births in the United States (2).

The advent of assisted reproductive techniques has accounted for much of the increase noted in rates of multiple gestations in recent years, with the trend towards an increased age of childbearing also a contributory factor. It is estimated that assisted reproductive techniques account for 10-24% of twin births (3). Meanwhile, the rate of twinning is increased 2.4 fold in women aged 35 – 39 years when compared to those aged below 20 years (3). In Ireland, the average age at childbirth has been steadily increasing over the past 2 decades, from 28 years in 1980 to 31.3 years in 2009, likely contributing significantly to the increased rates of multiple births seen.

Twin pregnancies are associated with worse perinatal outcomes than singleton pregnancies, with increased rates of preterm delivery among twin pregnancies and commensurate increases in all measures of perinatal morbidity and mortality (4-6). Rates of preterm delivery are increased in multiple pregnancies, firstly due to a higher likelihood of spontaneous preterm labour when compared to singleton pregnancy, and secondly, due to an increased chance of indicated preterm delivery
among twins secondary to maternal medical conditions or to intrauterine growth abnormalities.

A case control study of 1,253 twin pregnancies (7) evaluated the rates of antepartum complications associated with twin pregnancies, along with perinatal morbidity and mortality outcome measures, identifying a 2.5 fold increase in the risk of maternal hypertension antenatally when compared with a matched cohort of singleton pregnancies. Placental abruption was 3 times more frequent among twin gestations, and when compared to singletons, twins were of lower birth weight, and had lower Apgar scores and higher rates of perinatal death.

Although much of the increase in perinatal morbidity found in twin pregnancies is a direct consequence of the increased rates of preterm delivery, there is a well-established link between perinatal morbidity and growth discordance in twin pairs (8-10). Various studies have evaluated this phenomenon and attempted to define a threshold for percentage difference in birth weight in a twin pair above which worse perinatal outcomes are seen (11-13).
1.1.2 Growth Abnormalities in Twin Pregnancies

Growth abnormalities affecting twin gestations include the occurrence of intrauterine growth restriction (IUGR) affecting one or both twins. IUGR is usually defined as estimated fetal weight or birth weight below the 10th centile for gestational age, and in both twin and singleton pregnancies IUGR is associated with increased perinatal morbidity and mortality. IUGR has several possible aetiologies: intrauterine infection, chromosomal/structural abnormalities or uteroplacental insufficiency are common etiological mechanisms in twin and singleton pregnancies.

Factors unique to multiple gestations that may play a role in IUGR include twin-to-twin transfusion syndrome and unequal placental sharing in monochorionic twin gestations. It is also hypothesized that 'placental crowding' may be a factor in the decreased rates of growth observed in twin pregnancies, particularly at later gestations (14). This theory suggests that with advancing gestation the rate of placental growth slows in twin and higher order multiple pregnancies with a consequent decrease in the rate of fetal growth. This putative mechanism for abnormal growth in twins has been studied in monochorionic and dichorionic twin pregnancies. There is evidence that monochorionic placentas and dichorionic-fused placentas are more likely to give rise to growth restriction. It is proposed that dichorionic separate placentas are more likely to derive blood supply from separate areas of the uterus, thus leading to larger placental masses and improved fetal growth (15).

The majority of twin pairs grow at a similar rate – i.e. have concordant growth. Where growth in a twin pair is discordant, the risk of perinatal morbidity increases. Studies evaluating perinatal outcome in twins have reported excess mortality and morbidity at increasing levels of discordance. A large retrospective study of 1,318 twin pairs determined that growth discordance >20% in twins was associated with adverse neonatal outcomes, including low and very low birthweight, neonatal intensive care admission, neonatal oxygen requirement, and
hyperbilirubinaemia. These differences were present irrespective of gestational age at delivery and independent of small for gestational age status (8).

Similarly, another retrospective study of 9,590 twin pairs found that growth discordance levels of greater than 25% were associated with higher rates of perinatal and neonatal mortality, along with increased risk of 5 minute Apgar scores <7 (16).

Results from the prospectively assessed ESPRiT cohort of twin pregnancies has defined an inter-twin birthweight difference of 18% as significant in both monochorionic and dichorionic twins (12). In this study, perinatal mortality and adverse perinatal outcomes were assessed in a cohort of 977 twin pairs. After controlling for gestational age at delivery, gender, and growth restriction, the authors found that in dichorionic twin and monochorionic twins without twin to twin transfusion syndrome, perinatal morbidity and mortality increased above the 18% threshold for birth weight discordance. In this cohort all perinatal morbidity measures were more frequent in monochorionic than dichorionic twins.

The mechanisms of discordant growth in twin gestations are multiple and differ according to chorionicity. In monochorionic twins the likely underlying pathology is an unequal sharing of the placental territory, along with the presence of inter-twin vascular anastomoses within the placenta. There is some element of shared vasculature in all monochorionic twin pregnancies, but in the majority of cases the flow of blood is balanced between the two circulations. Twin to twin transfusion syndrome (TTTS) is a complication unique to monochorionic twin pregnancies, in which there is unbalanced unidirectional flow from a smaller ‘donor’ twin to a larger ‘recipient’ twin. Untreated it is associated with markedly increased morbidity and mortality (17). In up to 15% of monochorionic twin pregnancies there is an imbalance in the blood flow leading to the development of TTTS (18). This unequal flow leads to an over-perfused recipient with polyhydramnios, polycythemia, and cardiac overload. The
donor twin is often growth restricted with oligohydramnios. The mortality in this situation is very high for both donor and recipient twins, with up to 60 - 90% mortality rates in untreated cases (17, 19).

However, significant growth discordance remains common in monochorionic twin pregnancies in the absence of TTTS. In such cases the underlying aetiology may be an inequality in the proportion of the placental mass distributed to each twin. Studies evaluating placental sharing in monochorionic twins suggest that unequal placental sharing is associated with a significantly increased risk of discordant birthweight (20). Although MC twins are genetically identical and exposed to the same intrauterine environment there may be epigenetic differences in gene regulation which may account for divergent growth. A study of expression of genes related to angiogenesis in placentas of growth discordant MC twins showed upregulation of several genes in the placental territory of the smaller twin (21).

There may also be an association with abnormalities of umbilical cord insertion site. In fact, such anomalies may be the underlying reason that the placental mass is unequally distributed. Studies to date evaluating the role of cord insertion site in twins have shown conflicting results (22-24). However, these studies have generally been limited by their retrospective nature, and/or their relatively small size.

Abnormal growth in MC twins is complex with a diverse range of factors underpinning it. As our knowledge of the potential mechanisms at play grows, it increases our ability to target antenatal surveillance with the aim of decreasing morbidity and mortality in these pregnancies.

The aetiology of growth discordance in dichorionic twin pregnancies is less clear and has received far less attention in the literature. As many dichorionic twins are genetically distinct from one another there is an acceptance that a certain degree of growth discordance may be normal. However, these differences in genetic potential will only account for a
small proportion of cases and relatively low levels of discordance. Gender differences have been shown to account for approximately 4% of cases of discordant birthweight in dichorionic twins, with twins of concordant gender significantly more likely to have concordant birthweights than male/female twin pairs (25).

In addition, it must be recognised that ultrasound estimation of fetal weight is associated with an inherent risk of over- or underestimation. Therefore, a common explanation for minor degrees of growth discordance is measurement error. It is most commonly the upper twin that is smaller than the presenting twin suggesting that fetal location may have an influence on growth.

As in the monochorionic twin pregnancy, abnormalities of placentation may underlie the development of growth abnormalities in dichorionic twins. This must however be a distinct entity, as dichorionic placentas do not have any direct vascular connections. Abnormalities of placental cord insertion site are a potential pathological factor in dichorionic, as well as monochorionic pregnancies. However, as in the latter, the data published to date in this regard have been conflicting. Indeed, abnormal growth in a dichorionic twin pregnancy may be similar in aetiology to abnormal growth in a singleton pregnancy, with uteroplacental insufficiency selectively affecting one placenta of a dichorionic pair driving discordant growth. Several studies have addressed the issue of placental abnormalities in dichorionic twins. In a study by Eberle et al, 99 dichorionic twin placentas were evaluated, and placental vascular lesions were found at a higher frequency in lighter twins than heavier co-twins (26). Meanwhile, a report on the placental characteristics of a cohort of 288 dichorionic twins observed that placental weight was lower in twin pairs with significant birthweight discordance (23).

It is very important to recognise that discordant birthweight in dichorionic twins is frequently a pathological entity and it is well established that these pregnancies are associated with increased perinatal risks. Diverging
growth in a dichorionic twin pair must not be labelled as a normal phenomenon and such pregnancies require increased antenatal surveillance.

In summary, the published data suggest that placental examination may hold the key to improving our understanding of the underlying mechanisms of growth discordance in twin pregnancies.
1.2 Placental Pathology

1.2.1 Placental Development

The placental mass serves as the interface between the mother and fetus and has multiple critical functions during pregnancy, including respiratory gas exchange, nutrient transport, and elimination of fetal waste. It is therefore crucial that placental development proceeds in an orderly fashion to ensure maintenance of the pregnancy and normal fetal growth and development.

Placental development progresses through the stages of implantation and invasion, with subsequent growth and maturation of the villus architecture. There is concurrent adaptation of the uteroplacental circulation and development of the fetoplacental circulation.

**Early Placental Development**

Following fertilization, the blastocyst, composed of an outer trophoblast layer and an inner cell mass, implants in and invades the maternal endometrial epithelium. It is postulated that the orientation of the blastocyst as it implants may determine cord insertion site. Rotation of the blastocyst such that the embryonic pole, where the inner cell mass is located, is not directly at the site of implantation can lead to an abnormal placental cord insertion, such as marginal or velamentous insertion. A marginal cord insertion denotes insertion at the edge of the placental disc, while in a velamentous insertion the cord inserts directly into the membranes, remote from the placental disc. The outer trophoblast layer ultimately gives rise to the placenta and membranes, with the inner cell mass being the origin of the embryo and umbilical cord.

Following attachment to the uterine epithelium the blastocyst undergoes further differentiation. The polar trophoblast - the area of trophoblast overlying the inner cell mass - becomes the syncytiotrophoblast, capable
of invasion into the uterine epithelium. As the early embryo becomes embedded in the decidual stroma, the syncytiotrophoblast layer extends to surround the entire conceptus. The remaining trophoblast cells make up the inner layers and are now termed cytotrophoblast. These cytotrophoblast cells rapidly divide and fuse with the syncytiotrophoblast, leading to continuous expansion of the latter, which itself has no generative capacity. The syncytiotrophoblast cells are the only cells in direct contact with maternal tissues.

**Lacunar Stage**

Following invasion, the next stage of development is the lacunar stage, in which small vacuoles appear within the syncytiotrophoblast mass. This commences approximately 8 days post fertilization. These vacuoles coalesce to form a system of lacunae. The lacunae are separated by pillars of syncytiotrophoblast called trabeculae. This process extends over the entire blastocyst. By approximately Day 12 post-fertilization, the blastocyst has deeply invaded the endometrium and the uterine epithelium closes over the implantation site.

Cytotrophoblast cells from the chorionic plate - the area of the placental mass close to the developing embryo - penetrate through the trabeculae to reach the maternal surface of the placenta by day 15. These cells, termed the extravillous trophoblast (EVT), are responsible for invasion of the maternal vessels leading to development of the uteroplacental circulation. Following initial invasion of the endometrial stroma and capillaries, the EVT reaches the spiral arteries, invading the walls and entering the lumen of the vessels. As the muscular walls of the spiral arteries are destroyed, and the endothelium replaced by trophoblast, maternal circulation within the placenta commences.
Villous Growth and Differentiation

Concomitant with invasion of the maternal spiral arteries and development of the uteroplacental circulation, the development of the villous structure of the placenta occurs. Proliferation of cytotrophoblast and resultant syncytial fusion within the trabeculae leads to trabecular elongation. The trabeculae develop branches protruding into the lacunae, or intervillous spaces. These trabecular structures are the primary villi. They are transformed into secondary villi with invasion of mesodermal cells from the chorionic plate giving the villi a mesenchymal core.

Within this mesenchymal core, fetal capillaries develop from haemangioblastic progenitor cells. These fetal capillaries are first evident at Day 18-20 post fertilization, and their presence marks the transformation into tertiary villi.

Development of the fetal capillaries within the villi brings the maternal and fetal circulations into close contact. The maternal blood perfusing the intervillous spaces via the remodelled spiral arteries is separated from the fetal blood in the villous capillaries by the placental barrier. With progressive villous differentiation this placental barrier decreases in thickness, reducing the maternal-fetal diffusion distance from 50-100mm to just 4-5mm at term. This is crucial for the normal functioning of the placenta, with abnormalities in this process evident in various pathological states.

The process of villous differentiation in the placenta continues throughout gestation. As tertiary villi increase in numbers during pregnancy, there are relatively few primary or secondary villi evident in a term placenta. Growth and differentiation of tertiary villi sees their progression through the subclasses of mesenchymal villi, immature intermediate villi, stem villi, mature intermediate villi, and terminal villi.
Terminal villi develop from mature intermediate villi as longitudinal growth of capillaries exceeds longitudinal growth of villi. This leads to the capillaries becoming coiled and looped, and protruding as lateral projections from the villous tree into the intervillous spaces. These terminal villi are covered by a very thin layer of syncytiotrophoblast; thus the capillaries within the terminal villi are in very close contact with the maternal circulation. By term, the terminal villi provide a surface area of approximately 13m² for gas exchange. Normal development of this villous architecture is vitally important to ensure the adequate supply of nutrients to the developing fetus.

1.2.2 Abnormal Placental Development

It is well established that normal placental development underpins appropriate fetal growth in utero. The consequences of aberrations in this developmental process range from first trimester pregnancy loss, to third trimester complications including abruption, intrauterine growth restriction, preeclampsia, and intrauterine fetal demise (27). Placental abnormalities include global derangements in the architecture of the villous tree and fetoplacental vasculature, as well as discrete placental lesions such as infarctions or retroplacental haemorrhage.

Studies of placentas from pregnancies complicated by IUGR and pre-eclampsia have identified abnormal development of the villi and vasculature in these cases. It is thought that the primary abnormality is a reduction in uteroplacental blood flow due to defective trophoblast invasion, with a lack of the normal physiological adaptation of the maternal spiral arteries (28, 29). This may be apparent sonographically, with decreased flow in the uterine arteries when evaluated with Doppler ultrasound studies. The classic Doppler ultrasonographic finding in these pregnancies is a high-resistance uterine artery waveform with diastolic notching (30).
The consequent reduced perfusion of the intervillous space leads to maldevelopment of the villous tree. Placental histological examination reveals evidence of accelerated villous maturation and increased numbers of ‘syncytial knots’, representing premature aging of the placenta. Both volume and surface area of terminal villi have been shown to be reduced in the placentas of growth-restricted fetuses (31). This pathology is seen in a subset of singleton IUGR pregnancies and is termed ‘chorion regression’ (32).

True placental infarcts represent areas of placenta where the maternal vascular supply has been occluded. This spiral artery occlusion results in villous ischaemia. Although a small degree of peripheral infarction is a common finding, and may indeed be regarded as normal, there is evidence that central or multifocal infarction may affect the functional capacity of the placenta (33). The presence of placental infarcts has been associated with higher rates of small for gestational age infants and adverse perinatal outcomes (34, 35).

Retroplacental haemorrhage is caused by premature separation and is classically associated with an acute clinical presentation with abdominal pain, bleeding, and fetal compromise. However lesser degrees of placental separation may be not be clinically evident and may only be apparent at placental examination. It has been demonstrated that the presence of retroplacental haemorrhage at placental histological examination is associated with an increased incidence of intrauterine growth restriction (36). It has also been seen more commonly in the placentas of smaller twins in growth discordant pairs (26).

Histological examination of placentas evaluating these distinct pathological entities gives valuable information regarding placental development, placental function and the contribution of placental histological abnormalities to disordered fetal growth.
1.3 Stereology

1.3.1 Overview of Stereology

Stereology is the process of measuring the three-dimensional properties of an object from a two-dimensional section. It employs random sampling and estimation methods to derive quantitative data, including volumes, lengths, numbers, and surface areas of the specimen being studied.

The use of random sampling methods helps to eliminate bias and ensure adequate representation of the entire object being studied. The application of uniform random sampling, where the first sampling point is randomly selected and the position of the remaining sampling points is determined by a pre-determined interval, ensures even coverage across the entire organ, with all parts having an equal chance of selection (37).

The direction of sampling is also randomised to enable estimation of cell volume and surface area. This randomisation of sampling direction can be achieved by isotropic uniform random sampling or vertical sectioning. Isotropic uniform random sampling randomises directions in 3D space and is achieved by embedding tissue samples in spherical moulds and selecting directions of sectioning at random.

In contrast, vertical sectioning randomises sections in 2D space. This is the sampling method usually applied in stereological examination of the placenta. Vertical sections are randomly selected along a horizontal reference plane – in this case the chorionic plate of the placenta.

Random sampling of an organ produces two-dimensional slices, which are interrogated to provide estimates of three-dimensional quantities of the organ including lengths, volumes and surface areas. Randomly positioned and oriented lines and points are superimposed on the slices to enable measurement of these quantities.
In a cut section, volume-occupying structures, such as villi and capillaries, will appear as profile areas. Membranes, when sectioned, will appear as lengths in a 2D slice. A randomly positioned counting frame enables counting of numbers of capillaries and villi to derive an estimation of length.

Volume estimation is performed by superimposing randomly positioned points over the slice as the probability of a point hitting an object will be determined by its volume.

Randomly positioned and oriented lines superimposed over the slice will intersect with membranes proportional to the surface density of the object under study. If the volume of the organ is known this can be used to convert surface density into an absolute surface area of the membrane.

Use of these stereological sampling and estimation methods is an accurate, reliable and efficient means of studying placental structure and function (37).
1.3.2 Role of Stereology in Assessment of Placental Function

The use of stereology in the study of the placenta has been well described. It has been used as a tool to assess placental morphology in normal and complicated pregnancies (38).

Normal fetal growth and development is contingent upon adaptation and development of the placental structure and function to ensure adequate delivery of oxygen and nutrients to the developing fetus from the maternal circulation. As described above, this complex developmental process ultimately leads to a highly organised villous trophoblast with a large surface area, enabling adequate diffusion from the maternal circulation to the fetal circulation. Placental function is dependant on villous and capillary volume and the surface area available for participation in the diffusion of oxygen and nutrients. The ability of stereological techniques to reliably quantify these components makes it an ideal tool to study the placenta.

Normal placental growth and development was assessed using stereology in a cohort of 92 placentas from uncomplicated pregnancies between 10 and 41 weeks gestation (39). This study demonstrated the growth and development that occurs in the villous system as gestation progresses with significant increases in the volume of intermediate and terminal villi occurring due to a combination of elongation and thinning. Villous maturation was demonstrated with a progressive increase in capillary length throughout gestation, coupled with a relative decrease in the volume of stromal connective tissue. Capillary growth occurs by progressive elongation, with a biphasic pattern of angiogenesis demonstrated.

Stereology has been used as tool to assess placental morphology in pregnancies complicated by pre-eclampsia (PE) and IUGR, and also in maternal complications including anaemia, asthma and diabetes.
PE and IUGR are common complications of pregnancy associated with abnormal placental villous development and chronic uteroplacental hypoxia. Stereology has assessed the individual effects of these two conditions on placental morphology. In a study of 24 placentas from pregnancies complicated by PE with or without IUGR, significant changes in placental villi and vasculature were found only in those pregnancies complicated by IUGR (40). IUGR, irrespective of coexistent PE, was associated with a reduction in villous volume at all levels of villi, as well as reduced capillary volume and surface area.

Maternal diabetes mellitus is also associated with intrauterine hypoxia, and changes on placental morphology have been demonstrated including increased fetoplacental angiogenesis and greater capillarisation of villi. These changes are more pronounced in pre-gestational than gestational diabetes mellitus (41-44).

Stereology has proved a reliable and efficient tool for the assessment of the changes in placental structure and function associated with a variety of fetal and maternal pregnancy complications. Its role as an adjunct in the assessment of placental morphology in twin pregnancies has been poorly studied to date. It has the potential to provide valuable additional data in our investigations of the aetiology of growth disturbances in these pregnancies.
1.4 Aim of the Study

The primary aim of this study is to investigate the contribution of placental pathological factors to growth abnormalities in twin pregnancies. Variables from gross placental examination, histological examination and stereological assessment will be compared between twin pairs with concordant and discordant growth.

Placental variables will be correlated with antenatal sonographic variables in order to investigate the hypothesis that antenatal Doppler studies are predictive of placental pathology in discordant twin pregnancies. The impact of placental developmental abnormalities on perinatal outcomes in twin pregnancies will be evaluated.
Chapter 2 Materials and Methods

2.1 ESPRiT Study

2.1.1 Study Overview

The ESPRiT Study was a prospective observational study of twin pregnancies, carried out by the Perinatal Ireland Research Consortium. Perinatal Ireland is an all-Ireland research network, comprising maternal-fetal medicine specialists from 8 tertiary-level Obstetric units. The participating hospitals in the consortium are the Rotunda Hospital, Dublin, the National Maternity Hospital, Dublin, the Coombe Women and Infants University Hospital, Dublin, Cork University Maternity Hospital, Galway University Hospital, the Regional Maternity Hospital, Limerick, Our Lady of Lourdes Hospital, Drogheda and the Royal Maternity Hospital, Belfast.

The ESPRiT Study was designed to prospectively evaluate twin pregnancies, and had two main aims. It was primarily designed to evaluate growth in twin pregnancies, in order to identify an inter-twin growth differential that was significant in terms of perinatal morbidity and mortality outcome measures (12).

The study also aimed to assess the role of placental pathology in the aetiology of growth discordance in twins. Placental morphology was studied at macroscopic and microscopic levels. Stereological studies were used in dichorionic twins to further evaluate and quantify the microscopic findings. Placental findings were evaluated in the context of inter-twin birth weight discordance, growth restriction, and perinatal outcomes. The association of antenatal sonographic findings with placental pathological findings was also evaluated.
2.1.2 Study Design

Study Enrollment
The study was carried out in the eight participating centres in the Perinatal Ireland network. Recruitment commenced in May 2007 and completed in October 2009. With the aim of recruiting approximately 1000 structurally normal monochorionic and dichorionic twin pregnancies and following them for the duration of pregnancy the following inclusion and exclusion criteria were applied:

Inclusion Criteria
- Gestational age at time of enrollment between 11+0 and 22+0 weeks
- Both fetuses alive at time of enrollment
- Intact membranes and mother not in labour at time of enrollment

Exclusion Criteria:
- Aneuploidy in either twin
- Major structural abnormality in either twin
- Twin reversed arterial perfusion sequence
- Monoamniotic twin pregnancies

Patient recruitment was carried out by Perinatal Ireland research sonographers based in each centre, patients were provided with a detailed information pack, and written consent to participate was obtained. Local research ethics committee approval was obtained at each centre.

Patients consented to serial sonographic assessment throughout pregnancy, detailed placental examination following delivery, and longer-term paediatric outcome evaluation.
Sonographic Surveillance

At recruitment all subjects underwent sonographic assessment of chorionicity and dating. Pregnancies were classified as dichorionic or monochorionic based on ultrasound assessment of the placenta and membranes. Pregnancies with separate placentas or evidence of placental tissue extending into the base of the inter-twin membrane were classified as dichorionic, with monochorionicity assigned when a thin inter-twin membrane was visualized meeting a single placenta at right angles. Each fetus was recorded as ‘Twin 1’ or ‘Twin 2’ based on the proximity of the gestational sac to the cervix.

Dating of pregnancies was based on menstrual data and ultrasound findings. If menstrual dates were uncertain, or a discrepancy of 5 days or more existed between menstrual dates and ultrasound biometry, an ultrasound based estimated date of delivery was assigned.

All study subjects underwent a detailed anatomical survey between 18 and 22 weeks gestation. Finding of a major structural abnormality prompted exclusion from the study. At this scan the following variables were recorded:

- Biometric parameters:
  - Biparietal diameter (BPD)
  - Head circumference (HC)
  - Abdominal circumference (AC)
  - Femur length (FL)
- Placental umbilical cord insertion site
- Number of blood vessels in the umbilical cord
- Uterine artery Doppler measurement
All twins underwent two-weekly ultrasound surveillance from 24 weeks gestation. At each visit the following variables were recorded:

- Biometric parameters: BPD, HC, AC, FL
- Estimated fetal weight (EFW): Calculated from Hadlock’s Formula
- Amniotic fluid volume: Deepest vertical pool (DVP) in each sac
- Umbilical Artery (UA) Doppler
- Middle Cerebral Artery (MCA) Doppler
- Ductus Venosus Doppler: Performed in twins with evidence of inter-twin growth discordance or abnormal UA or MCA Doppler

In addition to the above scheduled scans, monochorionic twins underwent additional two-weekly surveillance from 16 to 24 weeks gestation, in order to monitor for the development of twin-to-twin transfusion syndrome.
2.1.3 Study Outcome Data

Detailed delivery and neonatal outcome data were collected for all study subjects.

The following delivery outcomes were recorded for each pregnancy:

- Gestational Age at Delivery
- Mode of Delivery for each twin
- Onset of Labour: Spontaneous/Induced
- Indication for induction of labour or pre-labour caesarean section (where applicable)
- Indication for intrapartum caesarean section or instrumental vaginal delivery (where applicable)
- Apgar scores for each twin
- Umbilical arterial and venous pH for each twin
- Birth weight for each twin
- Gender for each twin

The level of birth weight discordance was calculated for each twin pair by expressing the absolute differences in birth weight as a percentage of the birth weight of the larger twin.

Neonatal outcome parameters recorded for each twin were:

- Presence of intraventricular haemorrhage
- Presence of hypoxic ischaemic encephalopathy
- Radiological evidence of periventricular leukomalacia
- Necrotising enterocolitis
- Respiratory Distress Syndrome
- Sepsis
- Days of ventilator use
- Congenital anomaly
- Death prior to 28 days of life, including cause of death
- Length of neonatal intensive care unit stay
2.2 Placental Examination

Following delivery, all placentas were collected for examination. Placental examination took place in the pathology department of the delivery hospital, according to an agreed national protocol. Where there was no pathologist available locally to carry out the placental examination in accordance with the study protocol, the placenta was transferred to the Rotunda Hospital pathology department for evaluation. All placentas were examined by pathologists with extensive experience in perinatal pathology.

At delivery, each cord was labeled with one cord clamp for ‘Twin 1’ and two cord clamps for ‘Twin 2’. Study protocol dictated that the decision regarding formalin fixation of placentas was to be made at a hospital level, and would be determined by local clinical practices and pathological investigative needs. Placentas undergoing fixation were immersed in 10% phosphate buffered formalin for a minimum of 24 hours.

Gross placental examination was performed to evaluate umbilical cord vessel number and umbilical cord insertion site. The site of insertion of the cord into the placental disc was recorded as central, marginal, or velamentous. Marginal cord insertion was defined as cord insertion at the edge of the placental disc. Velamentous insertion denoted umbilical cord insertion into the membranes remote from the placental disc. All other cord insertions were recorded as central.

Evaluation of placental chorionicity was performed by examination of the inter-twin membrane. A sample was taken either from the junction of the inter-twin membrane and the chorial plate, or from the free inter-twin septum. Both gross and histological examination of the membrane was performed, and each twin pregnancy was recorded as dichorionic or monochorionic.
Prior to sampling of the placentas for histological examination, membranes were trimmed from the placental disc and the umbilical cords removed. In order to retain identification of the corresponding twin, the cord of twin 1 was cut flush with the placental disc, with that of twin 2 cut to leave 2-3 cm of cord attached to the disc.

The membranes were sampled at the rupture site and at a further random site, and examined histologically. Cross-sections of each umbilical cord from the placental end and the fetal end were submitted for microscopy. Each placental disc was serially sliced at 1–1.5 cm intervals. The plane of section was from fetal to maternal surface and at right angles to the axis of the placental equator in the case of conjoined dichorionic or monochorionic placentas. The following placental pathological lesions were recorded:

- Placental Infarction
- Chorangioma
- Subchorial Fibrin Deposition
- Retroplacental Haematoma

All pathological lesions identified were sampled for histological assessment. The size of the lesion was recorded, along with its location relative to twin 1 or twin 2.

Additional histological examination was carried out from 3 to 4 additional samples of placental parenchyma. In all cases it was attempted to examine full transmural sections from fetal to maternal surfaces.
2.3 Placental Stereology

2.3.1 Case Selection

The aim of the stereological arm of the study was to evaluate the hypothesis that discordant birth weight in dichorionic twin pairs may be attributed to chronic uteroplacental insufficiency selectively affecting one placenta only, with the value of antenatal sonographic findings in predicting placental pathological findings also under investigation.

Accordingly, cases for stereological examination were selected on the basis of birth weight discordance, placental histological findings, and/or antenatal sonographic findings suggestive of uteroplacental insufficiency. The following groups were defined at the outset of the study for stereological assessment:

1) DC twins with birth weight discordance $\geq 20\%$, with evidence of accelerated villous maturation in the placenta of the smaller twin, a larger twin with a histologically normal placenta, and a smaller twin with antenatal sonographic findings suggestive of uteroplacental insufficiency (defined as abnormal UA Doppler – PI $> 95^{\text{th}}$ centile or absent or reversed end diastolic flow, and/or oligohydramnios in the absence of ruptured membranes)

2) DC twins with birth weight discordance $\geq 20\%$, with evidence of maternal vascular malperfusion in the placenta of the smaller twin, and a larger twin with histologically normal placenta, but no abnormal antenatal sonographic findings

3) DC twins with birth weight discordance $\geq 20\%$, with both placentas histologically normal
4) DC twins with birth weight discordance ≤ 10%, with evidence of maternal vascular malperfusion in the placenta of one twin, and the other twin with a histologically normal placenta

5) Control twins: DC twins with birth weight discordance ≤ 10%, with both placentas histologically normal

6) Control singletons:
   a. 10 placentas from gestational age matched normal singleton pregnancies (representing normal uteroplacental perfusion)
   b. 10 placentas from gestational age matched singleton pregnancies complicated by pre-eclampsia and intrauterine growth restriction (representing impaired uteroplacental perfusion)
2.3.2 Stereological Examination

Placental Sampling

Following a review of the ESPRiT cohort, the relevant placentas were identified in accordance with the above groups for stereological examination. Placentas were retrieved from storage and transferred to the Pathology Department at the Rotunda Hospital, Dublin. The initial step was to record the weight and volume of the placental disc. The placental membranes had been trimmed from the disc, and the umbilical cord cut flush with the chorionic plate prior to weighing. The volume of each disc was measured using fluid displacement. A container with an overflow outlet was filled with water to the level of the outlet. The placenta was placed into the water, and the displaced fluid collected from the overflow outlet. The volume of displaced water was measured corresponding to the volume of the placental disc.

Placental sampling for stereology was then performed using the uniform random sampling method. This method reduces bias, as all parts of the placenta have an equal chance of being sampled. The principle of uniform random sampling mandates that the first sample be randomly selected, with subsequent samples chosen by a pre-determined pattern or interval. This allows uniform sampling across the entire placenta (37). In our study 10 samples were taken from each placenta. The first site was randomly chosen and then samples were taken at 10mm interval along the length of the placental disc. Each sampling site was marked with a pin, with a 1cm wide tissue sample subsequently excised from each sampling point. The samples were full-thickness from fetal to maternal surface of the placental disc. Each sample was then bisected and placed in a cassette labeled with the study number and twin identity (i.e. twin A or twin B). In order to increase the efficiency of the study, it was decided to reduce the number of samples from 10 to 5 for each placenta. One half of each bisected sample from samples 1 – 5 was replaced by a half of each sample 6 – 10, Samples 1- 5 were retained for study. Thus the number of samples was
reduced, while the area of the placenta sampled remained the same. The cassettes were then transferred to the histology department of the Rotunda Hospital, Dublin for processing.

**Tissue Processing**

The tissue samples were processed, sectioned, and stained by the staff of the histology laboratory according to a defined protocol to produce paraffin embedded, haematoxylin and eosin (H&E) stained, microscopic slides. The process was performed using the VIP Tissue-tek processor. Initially the samples were dehydrated by exposure to industrial methylated spirits for varying lengths of time. This was followed by immersion in chloroform and finally embedding in paraffin wax.

Once embedded in wax blocks, the tissue samples were sectioned using a microtome. 4 µm thick sections were cut as per standard histology sectioning protocol. A sample was selected, mounted on a glass slide and allowed to dry in preparation for staining. Prior to staining with H&E, the sections were de-waxed and rehydrated. This process was performed using the VIP Tissue processor.

Following this tissue preparation process, each placenta was represented by 5 slides each containing 2 tissue sections. The analyst performing the stereological examination was blinded to the clinical outcome.

**Stereological Examination**

All stereological estimates were obtained using the digital Histometrix 6.0 software (Kinetic imaging Ltd). Slides were visualized using a Nikon Eclipse E600 light microscope and a JVC 3-CCD colour video camera interfaced with an IBM personal computer. Stereology was performed on all slides using the Histometrix software at 40x magnification and measurements were made on digitized images of the terminal villi and capillaries (Fig. 2.1 and Fig. 2.2). A single Histometrix protocol was
designed for this study. The protocol was designed to estimate the volume and surface area of the terminal villi and capillaries. A total of 50 fields were analysed for each protocol, therefore one hundred fields were analysed per placenta.

**Figure 2.1** Stereological examination of terminal villous volume using *Histometrix 6™* software
Figure 2.2  *Stereological examination of terminal villous surface area using Histometrix 6™ software*

For each case included in the stereology study the following variables were obtained:

- Terminal villous volume
- Terminal villous surface area
- Capillary volume
- Capillary surface area

For volume estimates, the stereologically obtained value was multiplied by the placental volume to obtain the total volume of terminal villi and capillaries.
2.4 Statistical Analyses

Statistical analyses were performed using SAS software (Version 9.1). Relative frequencies were compared using chi-squared test. Analysis of variance (ANOVA) and paired student t tests were used to analyze continuous variables. Odds-ratio and 95% confidence intervals were used to estimate risks and the uncertainty around the risk estimates. A p value of <0.05 was considered statistically significant.
3.1 Introduction

Examination of the gross placental specimen following delivery yields valuable information with respect to placental developmental abnormalities and thus forms part of routine post partum procedures in all deliveries.

In twin pregnancies placental examination must be performed in order to confirm placental chorionicity. Chorionicity is initially established on antenatal ultrasound. With the attendant increases in complications found in monochorionic twin pregnancy, accurate assessment of chorionicity is vital in risk stratification and planning antenatal sonographic surveillance schedules. Studies have shown that twins sharing a placenta have a 3 - 6 fold increase in perinatal complications, including preterm delivery, growth abnormalities, intrauterine demise, and TTTS (4, 45, 46). Thus, more intensive antenatal surveillance is warranted in these pregnancies. Ultrasound evaluation of chorionicity is best performed at 10 – 14 weeks gestation. Previous studies evaluating the accuracy of ultrasound in correctly predicting placental chorionicity have determined the overall accuracy to range from 77.3% - 100% (47-52).

Placental umbilical cord insertion site has been evaluated as a contributory factor to perinatal morbidity in singleton and twin pregnancies. In singleton pregnancies, a velamentous cord insertion is associated with obstetric complications including prematurity, congenital anomalies, and fetal growth restriction (53, 54). A velamentous insertion is found in approximately 1% of singleton pregnancies, with an additional 7% of singletons having a marginal cord insertion (55).

Non-central cord insertion is more common in twin than in singleton pregnancies (55). In particular, monochorionic twin pregnancies have significantly higher rates of velamentous cord insertion (56). This has
been proposed as a contributory factor in the development of selective intrauterine growth restriction in monochorionic twin pregnancies, with studies showing conflicting results with respect to the role of velamentous cord insertion in the aetiology of twin-to-twin transfusion syndrome (57, 58).

Single Umbilical Artery (SUA) is noted in 0.2 – 1.1% of singleton pregnancies (50), and is a finding that generally prompts increased antenatal surveillance due to the association with a range of pregnancy complications. The rates of intrauterine fetal demise are higher in these pregnancies and there is also an increased likelihood of intrauterine growth restriction (59-61). Studies evaluating SUA in twin pregnancies have consistently reported a higher rate compared with singletons (62). However, there is a wide variation in the reported rates, and many of the studies have reported on high risks cohorts of twin pregnancies wherein SUA may be overrepresented. It is well established that umbilical cord abnormalities, including SUA, are more frequently encountered in pregnancies conceived using assisted reproductive techniques (ART), which may explain some of the increase in incidence of SUA observed among twins (63).

The cohort of twins recruited to the ESPRiT study represents a large unselected group of structurally and chromosomally normal twins. Therefore, they provide an ideal cohort in which to determine the true incidence of SUA and its possible association with adverse outcomes in twin pregnancies.
3.2 Aim

The aim of this arm of the study was to evaluate gross placental pathological findings and to identify associations between placental structural abnormalities and disordered growth in utero in twin pregnancies. The impact of chorionicity on any such associations was also evaluated.

3.3 Methods

The present analysis involved gross placental examination from twin pregnancies enrolled in the ESPRiT study. The rationale behind, and methods used in, the ESPRiT study are discussed in detail in chapter 2. Briefly, placental examination was performed in the pathology department of the delivery hospital, or performed in the pathology department of the Rotunda hospital in cases where appropriate pathology services were not available in the delivery hospital.

Gross placental findings recorded were placental chorionicity, placental cord insertion site (central, marginal or velamentous), and cord vessel number (3 vessel cord or 2 vessel cord). These findings were correlated with perinatal outcomes including birth weight, inter-twin birth weight discordance, and small for gestational age status. Gross placental outcome data was also correlated with antenatal sonographic findings; these results are presented in Chapter 4.

All twin pregnancies recruited to the ESPRiT study had sonographic determination of chorionicity performed at the first ultrasound assessment. In the majority of cases this occurred in the first trimester. Pregnancies were classified as dichorionic if there were two separate placental masses, if the gender was discordant, or in the case of a single placental mass, based on the presence of a thick inter-twin membrane with evidence of chorionic tissue extending into the base of the inter-twin membrane (the "lambda' sign). Sonographic findings suggestive of
monochorionic placentation were concordant gender, a single placental mass, and a thin inter-twin membrane meeting the placenta at right angles (the ‘t’ sign).

Placental examination therefore was performed by pathologists, either at the delivery hospital or in the Rotunda hospital. The candidate did not perform placental examination, but designed and planned the present analysis, and analysed and reported the associated data. The contributions of pathologists participating in ESPRiT have been acknowledged in this thesis and in the resulting published manuscripts.

3.4 Results

One thousand and twenty eight twin pregnancies were recruited to the ESPRiT study across eight tertiary level centres in Ireland. 27 patients were lost to follow up, with 1001 completing the study and delivering at one of the eight participating centres.

In 165 cases no placental examination was performed, either due to failure to retain the placenta for pathological examination following delivery, or due to lack of an appropriately trained pathologist to perform the examination. The remaining 836 placentas had a minimum of a gross placental examination performed.
3.4.1 Placental Chorionicity

Of the 836 placentas submitted for pathological examination, 820 had chorionicity evaluated and documented. These consisted of 167 monochorionic twin pregnancies (20.4% of the cohort) and 653 dichorionic twin pregnancies (79.6% of the cohort). In the remaining 16 cases, the chorionicity was either not recorded or was unable to be determined by the pathologist.

Evaluating the association between chorionicity and clinical outcomes (Table 3.1), monochorionic (MC) twins were of lower mean birth weight than their dichorionic (DC) counterparts (MC: 2204g; DC: 2520g; p<0.0001), and delivered at an earlier mean gestational age (MC: 34.7 weeks; DC: 36.3 weeks; p<0.0001).

Although MC twins were of lower mean birth weight, there was no significant difference in the proportion of MC twins that had a birth weight below the 5th centile for gestational age. (MC: 5.8%; DC: 7.0%; p=0.5). Birth weight discordance, an established risk factor for perinatal morbidity, was evaluated at the 10% and 20% level. Approximately half of all twin pairs had birthweight that differed by 10% or more. More significant birth weight discordance of 20% or greater occurred in 17.3% of the overall cohort. When stratified by chorionicity, there was a trend to increased rates of significant discordance among monochorionic twins, but this was not found to be statistically significant.

Perinatal morbidity was higher among MC twins, with one or more of the morbidity outcomes included in the composite perinatal morbidity present in 29.3% of MC and 14.1% of DC twins.
Table 3.1: Chorionicity and Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Monochorionic</th>
<th>Dichorionic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>167 (20.4)</td>
<td>653 (79.6)</td>
<td></td>
</tr>
<tr>
<td>Birthweight</td>
<td>2204 (646)</td>
<td>2520 (563)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>34.7 (3.1)</td>
<td>36.3 (2.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BW Disc. &gt;10%</td>
<td>53.3%</td>
<td>49.8%</td>
<td>0.14</td>
</tr>
<tr>
<td>BW Disc. &gt;20%</td>
<td>19.8%</td>
<td>16.7%</td>
<td>0.36</td>
</tr>
<tr>
<td>BW &lt;5th centile</td>
<td>5.8%</td>
<td>7.0%</td>
<td>0.5</td>
</tr>
<tr>
<td>Perinatal</td>
<td>49 (29.3)</td>
<td>92 (14.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Morbidity

Data are expressed as mean (standard deviation);
GA: Gestational age at delivery; BW Disc.: Birthweight discordance;
Perinatal morbidity: Composite of death, respiratory distress syndrome,
hypoxic-ischaemic encephalopathy, intraventricular haemorrhage,
periventricular leukomalacia, necrotizing enterocolitis, sepsis

Ultrasound Evaluation of Chorionicity

Placental pathological examination determined chorionicity as previously
described. The results of the placental examination and the sonographic
assessment were compared to determine the sensitivity of sonographic
determination of chorionicity.

Of 820 placentas with chorionicity assigned at placental histological
examination, sonographic assessment was correct in 95.5% (n=783) of
cases.

Table 3.2 demonstrates the accuracy of sonographic assessment of
chorionicity stratified into monochorionic and dichorionic cohorts. Overall,
86.2% of monochorionic and 97.9% of dichorionic twin pregnancies were
correctly predicted by antenatal ultrasound. Table 3.3 illustrates sensitivity and specificity of antenatal sonography for the determination of monochorionicity.

Table 3.2: Ultrasound Assessment of Chorionicity

<table>
<thead>
<tr>
<th>Ultrasound Diagnosis</th>
<th>Placental Pathology</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monochorionic</td>
<td>144</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Dichorionic</td>
<td>23</td>
<td>639</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>167</td>
<td>653</td>
</tr>
</tbody>
</table>

Table 3.3: Sonographic prediction of monochorionicity

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86.2%</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.9%</td>
</tr>
<tr>
<td>PPV</td>
<td>91.1%</td>
</tr>
<tr>
<td>NPV</td>
<td>96.5%</td>
</tr>
</tbody>
</table>

*PPV: Positive Predictive Value; NPV: Negative Predictive Value*
3.4.2 Placental Cord Insertion Site

Of the 836 placentas submitted for pathological examination, 816 had the placental cord insertion documented. In the remaining 20 cases, the umbilical cord insertion site was impossible to determine or was not recorded. The clinical characteristics of the 816 patients are outlined in Table 3.4. The findings are similar to the cohort outlined above. There were 13 monochorionic twin pregnancies affected by twin-to-twin transfusion syndrome, representing 7.8% of monochorionic twins.

Table 3.4: Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Monochorionic</th>
<th>Dichorionic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number</td>
<td>165 (20.2)</td>
<td>651 (79.8)</td>
<td></td>
</tr>
<tr>
<td>Mean BW (g)</td>
<td>2207 (647)</td>
<td>2517 (565)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean GA (wks)</td>
<td>34.7 (3.1)</td>
<td>36.3 (2.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BW Disc.&gt;20%</td>
<td>32 (19.4)</td>
<td>109 (16.7)</td>
<td>0.42</td>
</tr>
<tr>
<td>BW &lt;5th centile</td>
<td>19 (5.8)</td>
<td>91 (7.0)</td>
<td>0.5</td>
</tr>
<tr>
<td>TTTS</td>
<td>13 (7.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as N (% of total) or mean (SD); BW: Birthweight; GA: Gestational age at delivery; TTTS: Twin-to-twin transfusion syndrome

Overall, 20% (n=327) of the 1632 individual twins in the study had non-central cord insertion demonstrated on placental pathological examination. Rates of marginal and velamentous cord insertion were 15.3% (n=250) and 4.7% (n=77), respectively. When the results were stratified by chorionicity, monochorionic twin pregnancies had significantly higher rates of non-central cord insertion when compared to dichorionic twin pregnancies (Odds Ratio 2.2, 95% Confidence Interval 1.7 – 2.9). The difference was significant for both marginal and velamentous cord insertion sites (table 3.5).
Table 3.5: Placental Cord Insertion Site: Relative Frequency according to Chorionicity

<table>
<thead>
<tr>
<th></th>
<th>Monochorionic</th>
<th>Dichorionic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>226 (68.5)</td>
<td>1079 (82.9)</td>
<td></td>
</tr>
<tr>
<td>Non-central</td>
<td>104 (31.5)</td>
<td>223 (17.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Marginal</td>
<td>73 (22.1)</td>
<td>177 (13.6)</td>
<td>0.0068</td>
</tr>
<tr>
<td>Velamentous</td>
<td>31 (9.4)</td>
<td>46 (3.5)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as N (% of total)

Non-central cord insertion sites were significantly more common in the smaller twins of birthweight discordant pairs when compared to both their larger co-twins and twins with concordant birth weights (p=0.0025) (figure 3.1). Birthweight discordance was significantly associated with both marginal (p = 0.03) and velamentous cord insertion (p = 0.003) when evaluated within the entire cohort.
Within the cohort of 165 monochorionic twin pregnancies, there were 32 cases of significant birthweight discordance (19.4%). Of these, 50% (n=16) of the smaller twins from birthweight discordant pairs had a non-central umbilical cord insertion. Velamentous cord insertion was documented in 9.4% of monochorionic twins and was significantly more frequent (21.9%) in smaller twins of birthweight discordant pairs than in larger co-twins or concordant controls (9.3% and 7.9% respectively, p = 0.007) (figure 3.2).
Of the 165 pairs of monochorionic twins in the study cohort, there were 13 cases of TTTS, a rate of 7.8%. There was no difference in the frequency of marginal or velamentous cord insertion in monochorionic twins with TTTS when compared to those without TTTS (table 3.6). Marginal cord insertions were found 19.2% (n=5) and 22.8% (n=68) of TTTS and non-TTTS cases, respectively. The frequency of velamentous cord insertion was 7.7% (n=2) for those with TTTS, compared to 9.7% (n=29) for those without. The distribution of non-central cord insertion site in those twins with TTTS was equal between donor and recipient twins.
Table 3.6: Frequency of non-central cord insertion in twin-to-twin transfusion syndrome

<table>
<thead>
<tr>
<th></th>
<th>TTTS</th>
<th>No TTTS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>13</td>
<td>152</td>
<td></td>
</tr>
</tbody>
</table>

**Placental Cord Insertion**

- **Central**
  - Marginal: 19 (73.1) vs. 207 (68.1), p = 0.76

- **Velamentous**
  - 5 (19.2) vs. 68 (22.4), p = 0.9
  - 2 (7.7) vs. 29 (9.5), p = 0.75

*Data are expressed as N (% of total); TTTS: Twin to twin transfusion syndrome*

In dichorionic twin pregnancies, there was a trend toward increased rates of non-central placental cord insertion among smaller twins (p=0.051). The frequency of marginal cord insertion in smaller twins was 18.3%, compared to 15.6% in larger co-twins, and 12.9% in the cohort with concordant birthweight (p=0.10). Velamentous cord insertion was observed in 5.5% of smaller birthweight discordant twins, 2.8% of larger birthweight discordant twins, and 3.4% of concordant twins (p=0.18).

*(Figure 3.3)*
Figure 3.3: Cord insertion Site; Dichorionic Twins

Lighter twins of discordant pairs compared to concordant twins and heavier twins of discordant pairs

The association between non-central cord insertion and intrauterine growth restriction was evaluated comparing frequencies of central, marginal, and velamentous cord insertion on those twins that were SGA and those that were AGA *(table 3.6)*. Overall, 57.9% (n= 11) of SGA monochorionic twins had a non-central cord insertion site. Statistically higher frequencies of both marginal and velamentous cord insertions were found in SGA monochorionic twins when compared to appropriately grown infants. Within the dichorionic cohort, no association was found between cord insertion site and SGA status.
Table 3.7: Association between Cord Insertion Site and growth restriction

<table>
<thead>
<tr>
<th></th>
<th>AGA</th>
<th>SGA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monochorionic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marginal</td>
<td>66 (21.2)</td>
<td>7 (36.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Velamentous</td>
<td>27 (8.7)</td>
<td>4 (21.1)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Dichorionic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marginal</td>
<td>163 (13.4)</td>
<td>14 (15.5)</td>
<td>0.54</td>
</tr>
<tr>
<td>Velamentous</td>
<td>41 (3.4)</td>
<td>5 (5.5)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data expressed ad N (% of total). AGA: appropriate for gestational age; SGA: small for gestational age.

Evaluating the risk of birthweight discordance for a fetus with non-central cord insertion gave an overall odds ratio (OR) of 1.8 (95% CI 1.2 – 2.6). This risk increased with monochorionicity (OR 2.4, 95% CI 1.1 – 5.0), and with the combination of monochorionicity and a velamentous cord insertion (OR 3.5, 95% CI 1.3 – 9.4) (table 3.7). The odds ratio for birthweight below the fifth centile for gestational age in monochorionic twins with a non-central cord insertion was 3.22 (95% CI 1.26 – 8.27). A velamentous cord insertion further increased this OR to 4.03 (95% CI 1.14 – 14.3).
Table 3.8 Risk of birthweight discordance and small for gestational age status according to cord insertion site

<table>
<thead>
<tr>
<th>Cord Insertion Site</th>
<th>Birthweight Discordance</th>
<th>Birthweight &lt;5&lt;sup&gt;th&lt;/sup&gt; centile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>All Twins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Non-central</td>
<td>1.8</td>
<td>1.2 – 2.6</td>
</tr>
<tr>
<td>Marginal</td>
<td>1.6</td>
<td>1.1 – 2.5</td>
</tr>
<tr>
<td>Velamentous</td>
<td>2.5</td>
<td>1.3 – 4.6</td>
</tr>
<tr>
<td>Dichorionic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Non-central</td>
<td>1.6</td>
<td>0.9 – 2.5</td>
</tr>
<tr>
<td>Marginal</td>
<td>1.5</td>
<td>0.9 – 2.6</td>
</tr>
<tr>
<td>Velamentous</td>
<td>1.8</td>
<td>0.7 – 4.4</td>
</tr>
<tr>
<td>Monochorionic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Non-central</td>
<td>2.4</td>
<td>1.1 – 5.0</td>
</tr>
<tr>
<td>Marginal</td>
<td>1.8</td>
<td>0.8 – 4.4</td>
</tr>
<tr>
<td>Velamentous</td>
<td>3.5</td>
<td>1.3 – 9.4</td>
</tr>
</tbody>
</table>

<sup>1</sup>Reference value
3.4.3 Umbilical Cord Vessel Number

The number of vessels in the umbilical cord was recorded at placental examination for 801 twin pairs in the cohort. Of the 1602 placentas a single umbilical artery (SUA) was present in 1.1% (n=18). While SUA was more common among MC twins (2.04%) than DC twins (0.92%), this finding was not statistically significant (p = 0.12).

Evaluating the correlation between the finding of a SUA and an abnormal placental cord insertion site, it was noted that umbilical cords with a marginal or velamentous insertion were more likely to have a SUA than those that had a central insertion (2.57% vs. 0.77%; p = 0.01).

The hypothesis that SUA was a contributory factor to inter-twin growth discordance was tested at the 10% and 20% level. An intertwin birthweight differential of 10% or greater was found in 44.5% of cases where one twin had a SUA. This was similar to twin pairs where both had a three vessel cord, with 48% of this group exhibiting >10% birthweight discordance.

A more significant degree of birthweight discordance of 20% or greater was present in 27.8% of twin pairs with a SUA in one twin. While this was more frequent than twins with three vessels in both umbilical cords, where 16.7% demonstrated >20% discordance, this finding did not reach statistical significance (p=0.2).
3.5 Discussion

Twin pregnancies pose a unique challenge in obstetric management, and are associated with markedly increased rates of perinatal morbidity (7). This is particularly true of MC twin pregnancies (64). Growth discordance is independently associated with adverse outcomes (8), and identification of factors that contribute to growth abnormalities in these pregnancies may improve antenatal risk stratification, with institution of intense fetal surveillance in high-risk cases.

Accurate determination of chorionicity is central to management of twin pregnancies. MC twin pregnancies demand a more intensive regimen of antenatal surveillance given the 2-fold increase in perinatal mortality associated with monochorionicity. In addition, MC twins are at risk of the unique complications of twin-to-twin transfusion syndrome and selective intrauterine growth restriction. In contrast to DC twin pregnancies, single twin demise in a MC twin pair poses significant risks to the surviving twin. A recent meta-analysis estimated the risk of co-twin death to be 15% in these situations, with 26% of surviving co-twins showing evidence of neurodevelopmental impairment (65).

These unique risks mandate a minimum of two-weekly sonographic surveillance in MC twin pregnancies, with increased surveillance in complicated MC twins. In contrast, the recommended frequency of antenatal sonographic evaluation in uncomplicated DC twins is four-weekly.

In addition to planning antenatal surveillance, knowledge of chorionicity informs decision-making regarding timing of delivery. Uncomplicated DC twins can progress to 38 – 39 weeks gestation. However, the majority of experts would recommend delivery in MC twins at 36 - 37 weeks, even in uncomplicated cases.
Previous studies reporting on sonographic prediction of chorionicity have reported up to 100% accuracy when chorionicity is assigned in the first trimester. It is well established that this accuracy decreases with advancing gestation (47-51).

Within our cohort, the accuracy of ultrasound was 95.5% when compared to definitive chorionicity assigned on placental histological examination. It was slightly lower in the MC cohort, and the overall sensitivity and specificity for sonographic determination of monochorionicity was 86.2% and 97.9%, respectively. This correlates well with the findings of Lee et al (47), who reported on a cohort of 410 twin pregnancies, in which chorionicity was determined sonographically prior to 24 weeks gestation, with 95.6% of cases of chorionicity correctly assigned. The 99.8% accuracy reported by Dias et al (50) represents a cohort of 648 twin pregnancies with first-trimester assessment of chorionicity. The slightly lower accuracy levels within our cohort reflect the fact that twin pregnancies were recruited to the ESPRiT study at booking gestations of up to 22 weeks. This finding reinforces the importance of early identification of twin pregnancies in order to accurately assign chorionicity, and thus plan antenatal surveillance schedules and peripartum management.

There are multiple putative mechanisms for discordant growth in twin pregnancies, which differ according to chorionicity. Studies evaluating placental factors have suggested that unequal placental sharing and abnormalities of umbilical cord insertion contribute to growth discordance in monochorionic twin pregnancies. An association with non-central umbilical cord insertion and birthweight discordance in dichorionic twins has been less consistently reported (22, 23, 56, 57, 66, 67). The majority of studies published have been retrospective, with any prospective studies published only evaluating risk in monochorionic twins.

In a retrospective analysis of 447 twin pairs, Hanley et al (66) found velamentous cord insertion did not predict birthweight discordance in
dichorionic twins, but was associated with a 13-fold increase in risk of discordance among monochorionic twins. Conversely, Victoria et al retrospectively evaluated 382 twin pregnancies with respect to growth discordance and placental pathology (23). Umbilical cord abnormalities - defined as velamentous cord insertion and single umbilical artery - were compared between concordant, mildly discordant, and severely discordant twin pairs. In both dichorionic and monochorionic pregnancies, umbilical cord abnormalities were significantly more frequent in the smaller twins of severely discordant twin pairs than in mildly discordant or concordant twin pairs.

These retrospective studies evaluating the impact of cord insertion site are supported by two prospective studies in monochorionic twins. De Paepe et al evaluated placental characteristics in 216 monochorionic twins without twin-to-twin transfusion syndrome. Higher rates of velamentous cord insertion were documented in the 36 birthweight discordant pairs in this study (22). The 22% rate of velamentous cord insertion found in birthweight discordant monochorionic twins in this study was comparable to the rate of velamentous cord insertion in smaller twins of birthweight discordant pairs in our study. This study also reported significantly increased frequencies of unequal placental sharing in birthweight discordant twins. This provides a putative explanation for the mechanism of birthweight discordance in the presence of an abnormal placental cord insertion.

Machin evaluated 60 monochorionic placentas and found marginal or velamentous cord insertion to be associated with an increased risk of significant birthweight discordance (68). The rate of velamentous cord insertion in the cohort studied by Machin was extremely high at 45%. The fact that this is significantly higher than rates found in our study and in other published studies indicate that the study sample may not have been representative of the general monochorionic twin population. While the results of the study by Machin support the findings of our study, our
study represents an unselected cohort of twins, and thus our results should be applicable to any population of twin pregnancies.

We performed a large, multi-center, prospective study of 135 monochorionic and 651 dichorionic twin pairs, and found abnormal placental umbilical cord insertion to be a significant predictor of discordant growth. We documented high rates of both marginal and velamentous cord insertions in twin pregnancies. Compared to reported frequencies of non-central cord insertion in singleton pregnancies (55), marginal cord insertion was noted approximately twice as often (15.3% vs. approx. 7%) in twin pregnancies in our study, with velamentous cord insertion approximately 5 times more frequent (4.7% vs. approx. 1%).

The rates of velamentous cord insertion in our study are somewhat lower than the rates reported in a large cohort of twin placentas evaluated by Sato and Benirschke (69). In their cohort of 389 monochorionic twins, the rate of velamentous cord insertion was 12%, with 7.3% of the 780 dichorionic twins in their study demonstrating a velamentous cord insertion. The differences in rates in the two studies may be attributable to the fact that, as theirs was a retrospective study, Sato and Benirschke may have had a higher proportion of complicated twin pregnancies, as these placentas are more likely to be retained for pathological examination.

Overall, the risk of birthweight discordance of 20% or greater was 80% higher for those with a non-central cord insertion site. This increase was observed both in twins with a marginal cord insertion and those with a velamentous cord insertion site, although the risk of growth discordance was markedly greater with the latter. The excess of non-central umbilical cord insertions found in the smaller twins of birthweight discordant twin pairs in our study was driven by the very high rate of velamentous cord insertions in birthweight discordant monochorionic twins. However, we did not find a similar relationship between umbilical cord insertion and growth discordance in dichorionic twin pregnancies. Similarly, the
association between non-central cord insertion and growth restriction, as defined by birthweight below the fifth centile for gestational age, was only observed in monochorionic twin pregnancies.

The results of this study point to a role for velamentous cord insertion in the aetiology of growth restriction in monochorionic twin pregnancies. As there was no difference noted in the relative rates of marginal and velamentous cord insertion in TTTS and non-TTTS monochorionic twin pregnancies, it would appear that this effect is independent of the role of TTTS in birthweight discordance.

A study by Sato and Benirschke evaluated the role of fetal vessel thrombi in the aetiology of growth discordance in monochorionic twin pregnancies (69). They concluded that thrombi were observed more frequently in placentas with a velamentous cord insertion. The increased risk of fetal vessel thrombi in these cases may be a factor in the increased rates of birthweight discordance and fetal growth restriction observed in monochorionic twins with a velamentous placental cord insertion site. The association between velamentous cord insertion and unequal placental sharing, as studied by De Paepe et al (22), provides another possible explanation for the association we observed between velamentous cord insertion and birthweight discordance.

Several possible limitations to our study are acknowledged. The number of placentas that failed to undergo examination raises the possibility that there is an overrepresentation of complicated twin pregnancies in our sample. However the proportions of monochorionic and dichorionic twin pregnancies, as well as the rates of both birthweight discordance and SGA status, are comparable in the cohort of 816 twins reported here with the entire cohort of 1001 twin pregnancies that completed the ESPRiT study. The fact that all placental examinations were not carried out in a single laboratory is another potential source of bias. However the nature of the placental examination variable being studied here, cord insertion site, should limit any potential bias in this regard.
The utility of sonographic assessment in determining cord insertion site has been examined in singleton and twin pregnancies. Di Salvo et al compared prenatal sonographic examination and postnatal histopathological findings with respect to umbilical cord insertion in 38 singleton and 8 twin pregnancies (70). 49 of the 54 cord insertion sites were correctly delineated with ultrasound, with an overall sensitivity and specificity of antenatal ultrasound of 69% and 100% respectively. The ability of a combination of gray-scale and color Doppler ultrasound to identify velamentous cord insertion was prospectively evaluated by Sepulveda et al in 832 singleton pregnancies (71). They determined that velamentous cord insertion could be reliably determined with prenatal sonography, with successful visualization of the placental cord insertion site in 99% of cases. Unfortunately, there remains a paucity of prospective data evaluating the reliability of sonographic assessment of cord insertion in twin pregnancies, and further research is warranted in this area.

With respect to the occurrence of SUA in our twin cohort, we report contrasting results with those previously published in the literature. The reason that only one umbilical artery develops in certain pregnancies is debated. Numerous factors have been shown to be associated with an increased incidence of SUA. These include advanced maternal age, smoking, multiparity, presence of pre-existing medical conditions, and twin pregnancies. In a retrospective review of 203,240 fetuses and neonates by Murphy-Kaulbeck et al (59), the rate of SUA was 0.44%. This was an isolated finding in 81.9% of cases, with no other structural defect and no underlying chromosomal abnormality. The risk factors identified for isolated SUA in this cohort were smoking, drug use, presence of maternal antibodies, neurological disease, pulmonary disease, pre-existing diabetes, chronic hypertension, and twins. The adjusted odds ratio for SUA in the presence of a multiple pregnancy was 2.73 (95% CI 1.88 – 3.97).
A recent study by Klatt et al (62) retrospectively studied 174 twin pregnancies, 100 DC and 74 MC. The rate of SUA in their cohort was 9.8% of twin pregnancies or 5.2% of fetuses. In keeping with previous studies, the rate of SUA was higher in the cohort conceived with ART at 18.2%. There were higher rates of birthweight discordance found within their cohort in twin pairs where one twin had a SUA.

The findings of our study appear to contradict the findings of these previous studies. We have demonstrated a very low rate of SUA in an unselected cohort of twin pregnancies. The exclusion of twins with both structural and chromosomal abnormalities likely accounts for the differences between this cohort and those previously reported in the literature.

The very low numbers of twins with a SUA in our cohort made it difficult to draw any definite conclusions regarding impact on perinatal outcome. Although it did not reach statistical significance, there was a higher rate of SUA in the MC cohort (2.0%), and a trend was also seen towards increased rates of significant BW discordance when one twin of a pair had a SUA.

In summary, we report a large prospective study evaluating associations between gross placental structural findings and growth aberration in twin pregnancies. In this unselected cohort of structurally and chromosomally normal twins, monochorionicity was associated with earlier delivery and lower birthweight, and conferred an increased risk of perinatal morbidity when compared to DC pregnancies. Placental cord insertion site was found to be predictive of discordant growth in monochorionic twin pregnancies. Therefore, we would recommend that sonographic assessment of cord insertion site be considered in twin pregnancies, with the finding of a marginal or velamentous cord insertion prompting increased antenatal fetal surveillance. However, further prospective examination of the accuracy of antenatal ultrasound in this setting is warranted.
4.1 Introduction

The aetiology of birthweight discordance in twins has been extensively investigated. In monochorionic twins, differences are largely attributable to twin-to-twin transfusion syndrome, inequalities in distribution of placental mass between the two fetuses, and abnormalities in cord insertion site (22, 72). In dichorionic twins, there may be a difference in genetic growth potential in certain cases, but frequently growth discordance is a pathological entity leading to adverse neonatal outcomes (73).

Placental pathological examination at both a gross and microscopic level is useful in informing our knowledge of the aetiology of abnormal growth in both singleton and twin pregnancies, with a variety of placental pathological lesions implicated in intrauterine growth restriction (74). Studies in singleton pregnancies have shown that the presence of placental fibrin deposition, avascular villi, and villous infarcts were predictive of restricted fetal growth (75-77).

In twin pregnancies, the presence of infarction, placental abruption, villous fibrosis, chronic villitis, and intraplacental thrombi have previously been shown to be associated with aberrant growth (23, 26). Prior published studies have, however, been retrospective in nature and thus subject to bias, as it is likely that pregnancies with adverse outcomes are overrepresented. With a prospectively collected cohort of twins, the ESPRiT study therefore provided an ideal opportunity to evaluate the contribution of placental pathology to birthweight discordance and intrauterine growth restriction in MC and DC twin pregnancies.

Doppler velocimetry of the Umbilical Artery is routinely used in the surveillance of high-risk pregnancies. Changes in the UA waveform
reflect underlying changes in feto-placental perfusion. Normally, the low resistance in the placental circulation allows forward flow from fetus to placenta throughout the cardiac cycle. This gives rise to the characteristic UA waveform (*Figure 4.1*), with a systolic peak and diastolic nadir, but with positive flow maintained at all times. Uteroplacental insufficiency is typically associated, in singleton pregnancies, with a high risk of elevated impedance in the umbilical arteries with consequent changes in the UA Doppler waveform. An initial reduction in diastolic flow is followed by an absence of flow during diastole and ultimately reversed end diastolic flow (*Figure 4.2*).

*Figure 4.1: Normal UA waveform*

*Figure 4.2: Reversed end diastolic flow*

Changes in Doppler waveforms of other fetal and maternal vessels are also recognized in pregnancies complicated by uteroplacental vascular pathology. The middle cerebral artery (MCA) waveform displays increasing flow during the diastolic component of the cardiac cycle in growth restricted fetuses. This ‘brain-sparing’ effect reflects cerebral
redistribution of blood flow, as the fetal circulation preferentially perfuses the brain. This results in a decrease in the MCA pulsatility index (PI).

This ‘brain-sparing’ effect can also be evaluated by calculating the cerebroplacental ratio (CPR). This ratio quantifies the redistribution of cardiac output by dividing the pulsatility index of the MCA by the pulsatility index of the UA.

Serial evaluation of UA Doppler is used in both singleton and twin pregnancies as a surveillance tool to aid with timing of delivery. In high risk singleton pregnancies complicated by intrauterine growth restriction and preeclampsia, UA Doppler findings correlate with perinatal outcome. In a metaanalysis of Doppler ultrasound in high risk pregnancies, use of UA Doppler surveillance reduced antenatal admissions, labour induction, and perinatal deaths (78).

Both MC and DC twin pregnancies, with their higher risks of adverse perinatal outcome, warrant increased sonographic surveillance, and research has focussed on identifying optimal surveillance tools to decrease adverse outcomes. UA Doppler assessment has been shown to be a useful adjunct to sonographic assessment of fetal biometry in the prediction of growth discordance in twins (79). However, studies evaluating the clinical benefit of routine UA Doppler measurement in twins have shown conflicting results.

In a study by Gaziano et al (80), an abnormal UA Doppler in twins was associated with increased perinatal morbidity, an increase in stillbirth and delivery at an earlier gesational age. Giles et al studied UA Dopplers in 272 twin pairs, with the results of the Doppler studies incorporated into clinical decision making after the first 100 cases (81). This study concluded that addition of Doppler parameters into clinical management resulted in decreased perinatal mortality and decreased neonatal ICU admission without a change in gestational age at delivery. However, in a subsequent randomised controlled trial of ultrasound biometry versus UA
Doppler plus ultrasound biometry in twin pregnancy, there was no significant improvement in neonatal outcomes with the addition of routine UA Doppler surveillance (82). This randomized controlled trial was underpowered due to its small size and lack of stratification by chorionicity.

Prior studies have noted high rates of abnormal UA Doppler velocimetrics in MC twin pregnancies (83). However, rates of Doppler abnormalities and their clinical significance is less well studied in DC twins. In addition, the majority of the published literature focuses on cohorts of MC and DC twins complicated by growth restriction, twin-to-twin transfusion syndrome, and other obstetric issues. There is a paucity of data on overall rates of Doppler abnormalities and the association with clinical outcomes in unselected cohorts of twins.

As abnormalities in fetal Doppler studies are suggestive of underlying placental dysfunction, it would be expected that histological examination of the placenta should reveal histopathological abnormalities consistent with utero-placental insufficiency. Studies in singleton pregnancies complicated by fetal growth restriction have supported this hypothesis. Placentas from singleton IUGR pregnancies have shown an association between abnormal UA Doppler indices and an increase in syncytial knots, fibrin deposition, and villous infarcts (84, 85).

As will be presented in the results of this chapter, we demonstrated that placental histology in growth discordant dichorionic twins shows similar pathological lesions in the growth restricted twin to that seen in IUGR singletons. It would therefore be expected that similar correlations with Doppler abnormalities would be found. In contrast, monochorionic twin placentas did not show an excess of histopathological lesions in the placentas of growth restricted twins, reflecting the differing pathological processes at play in MC and DC growth discordance. Additionally, abnormal placental cord insertion site was only predictive of growth restriction in MC twins.
We therefore postulated that abnormal Doppler indices would be more likely to correlate with underlying placental histological abnormalities in the DC cohort. Conversely, we hypothesised that, an abnormal placental cord insertion was more likely to be associated with abnormal Doppler findings in MC twins.

4.2 Aim

The aim of this arm of the study was, firstly, to evaluate the contribution of abnormalities of placental histology to aberrant growth in twins. Secondly, we aimed to assess the frequency of abnormal Doppler studies on antenatal sonography, and to correlate Doppler findings with clinical outcome and placental pathology.
4.3 Methods

The analyses presented here included twin pregnancies enrolled in the ESPRiT study, which had undergone histological examination and Doppler ultrasound evaluation. As discussed in detail in chapter 2, placental histopathological analysis was performed at the delivery hospital, or in Rotunda hospital where appropriate expertise was not available locally. The presence on histological examination of placental infarction, chorangioma, subchorial fibrin, retroplacental haematoma, and/or abnormal villous maturation were recorded.

The relationship of placental histological abnormalities with chorionicity and birthweight discordance was evaluated, as was the association of placental histological lesions with antenatal Doppler abnormalities. Finally, the ability of Doppler ultrasound to predict adverse clinical outcomes was examined.

Pathologists either at the delivery hospitals or at the Rotunda hospital performed placental histological examination. Sonographers at the delivery hospital performed Doppler ultrasound. The analyses presented here were designed and planned by the candidate, who analysed and reported the relevant data.
4.4 Results

4.4.1 Placental Histology

Of 1001 twin pairs recruited to the ESPRiT study, 66.7% (n=668) had complete placental histopathological examination data available for analyses. Of these, 141 (n=21.1%) were monochorionic and 78.9% (n=527) dichorionic. Table 4.1 illustrates the clinical characteristics of the cohort. Monochorionic twins were delivered at an earlier mean gestational age and were on average 302 grams lighter than their dichorionic counterparts. The relative frequency of both BW discordance of 20% or greater and birthweight less than the 5th centile was not statistically significantly different between the two groups. A composite measure of adverse perinatal outcome, which included any of the morbidity measures described above or perinatal death, was more frequent in monochorionic twins.
Table 4.1: Clinical Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Monochorionic</th>
<th>Dichorionic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n (%)</td>
<td>141 (21.1)</td>
<td>527 (78.9)</td>
<td></td>
</tr>
<tr>
<td>Mean BW, g</td>
<td>2201 (632)</td>
<td>2504 (567)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean GA at delivery, wks</td>
<td>34.7 (2.9)</td>
<td>36.3 (2.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BW Discordance &gt;20%</td>
<td>26 (18.4)</td>
<td>88 (16.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>BW &lt;5th centile</td>
<td>16 (5.7)</td>
<td>76 (7.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>Composite perinatal morbidity</td>
<td>83 (29.4)</td>
<td>150 (14.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as n (% of total) or mean (SD)

BW: birthweight; GA: gestational age

Overall, 34.7% (n=464/1336) of twins in the study group had a placenta that demonstrated one or more of the placental pathological lesions (‘composite placental outcome’) assessed. Lesions were more frequently seen in the placentas of monochorionic than dichorionic twins (p=0.009, table 4.2). When the individual histological abnormalities were categorized, all abnormalities other than chorangioma were significantly more common in monochorionic placentas (table 4.2).
Table 4.2 Frequency of placental histological abnormalities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Monochorionic</th>
<th>Dichorionic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarction</td>
<td>34 (18.7)</td>
<td>88 (8.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chorangioma</td>
<td>0 (0)</td>
<td>7 (0.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Subchorial fibrin</td>
<td>32 (17.6)</td>
<td>113 (10.7)</td>
<td>0.012</td>
</tr>
<tr>
<td>Retroplacental haematoma</td>
<td>17 (9.3)</td>
<td>53 (5.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Abnormal villus maturation</td>
<td>53 (29.1)</td>
<td>151 (14.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Composite placental</td>
<td>117 (41.5)</td>
<td>347 (32.9)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Data are expressed as n (% of total)

The relationship between birthweight discordance and placental abnormalities was then analyzed. Overall, 17.1% (n=114) of the cohort had >20% difference in birthweight between the smaller and larger twin. The results for the smaller discordant twins (n=114) were compared to the combined group of larger co-twins and twins with concordant birthweight (n= 1222). 44.7% of the smaller twins had abnormal findings at placental examination. This was significantly more frequent than the comparison group of larger twins and twins with concordant birth weight (33.8%, p=0.02)(figure 1).

These results were then stratified by chorionicity to ascertain if this relationship was present in both monochorionic and dichorionic twins (figure 1). A significant association between abnormalities in the placenta and birthweight discordance was found in the dichorionic cohort, with a higher frequency of placental pathological lesions in the smaller twins, but a similar association was not observed in monochorionic twins.
A further analysis of the results was performed to determine the correlation between fetal growth restriction and placental pathology, comparing twins with birth weight below the 5th centile for gestational age (small for gestation, SGA) with those with birthweight above the 5th centile (appropriate for gestational age, AGA). Of the 6.7% (n=90) of SGA babies within the cohort, 57.8% (n=52) had evidence of one or more of the described abnormalities of placentation. This was almost twice as frequent as those that were AGA (n= 412/1246, 33.1%, p=0.0001). When analyzed separately according to chorionicity, the association between SGA status and placental pathology was significant in dichorionic, but not monochorionic twins (p=0.0001 and 0.23, respectively, figure 2), although the latter analysis was limited by the relatively low number of SGA monochorionic twins (n=16) in our study cohort.

Figure 4.3 Relative frequencies of placental histological abnormalities in BW discordant twin pairs
Figure 4.4 Relative Frequency of placental histological abnormalities in small and appropriate for gestational age twins
4.4.2 Antenatal Doppler Studies

All patients recruited to the ESPRiT study consented to serial ultrasound assessment including multi-vessel Doppler studies. At each sonographic examination, umbilical artery (UA) and middle cerebral artery (MCA) Doppler was performed. An abnormal UA Doppler was defined as a pulsatility index (PI) >95\textsuperscript{th} centile for gestation, or absent or reversed end diastolic flow. An abnormal MCA Doppler was defined as a PI <5\textsuperscript{th} centile for gestation. The cerebroplacental ratio (CPR) was then calculated as the ratio of the MCA PI to the UA PI. A CPR <1 was considered abnormal. In addition to these serial Doppler assessments, all patients consented to assessment of uterine artery (UtA) Doppler at 18 - 22 weeks. UtA PI >95\textsuperscript{th} centile was considered abnormal.

Of the 1001 patients recruited to the ESPRiT study, 94.4\% (n=945) had UA Doppler data recorded, and 93.2\% (n=933) had MCA Doppler findings available for analysis. There was significant variation in the recording of UA Doppler findings, and after exclusion of those cases with insufficient data or suboptimal image acquisition, just 13.8\% of cases had satisfactory data available on UtA velocimetreries to be included in the analysis.

The relative frequency of abnormal Doppler findings was analysed, evaluating both the proportion of twins with an abnormal Doppler at any time during pregnancy and those that had an abnormal Doppler at the final scan prior to delivery. (Table 4.3). Influence of chorionicity on the likelihood of an abnormal Doppler study was assessed.

Overall, 38.7\% (n=732) of twins had an abnormal UA Doppler recorded at some time during the pregnancy. This was not significantly higher in the MC cohort (39.5\% vs. 38.6\%; p=0.76). Regarding the final scan prior to delivery, 16.7\% of MC had an abnormal UA Doppler, which was not significantly higher than their DC counterparts (13.9\%; p = 0.20).
An MCA PI <5\textsuperscript{th} centile was found very frequently in both MC and DC twins (66.9\% and 66.5\% respectively), and approximately one-third of twins had an abnormal MCA prior to delivery. Again, chorionicity did not impact on the frequency of this finding.

The cerebroplacental ratio, calculated in 93.1\% of the overall cohort, was abnormal in 13.2\% of fetuses during at least one sonographic assessment and in 3.6\% at the final sonogram. The relative frequencies were similar in MC and DC twins.

Table 4.3: Frequency of abnormal antenatal Doppler findings; Comparison between monochorionic and dichorionic pregnancies

<table>
<thead>
<tr>
<th>Abnormal Doppler</th>
<th>Monochorionic</th>
<th>Dichorionic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Scan</td>
<td>39.5% (135)</td>
<td>38.6% (597)</td>
<td>0.76</td>
</tr>
<tr>
<td>Final Scan</td>
<td>16.7% (57)</td>
<td>13.9% (9215)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>MCA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Scan</td>
<td>66.9% (221)</td>
<td>66.5% (985)</td>
<td>0.89</td>
</tr>
<tr>
<td>Final Scan</td>
<td>37.6% (124)</td>
<td>35.4% (524)</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>CPR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Scan</td>
<td>15.2% (50)</td>
<td>12.7% (187)</td>
<td>0.24</td>
</tr>
<tr>
<td>Final Scan</td>
<td>3.7% (12)</td>
<td>3.5% (52)</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>UtA</strong></td>
<td>9.5% (4)*</td>
<td>10.3% (24)*</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are expressed as \% of total individual twins (N) or *\% of pregnancies (N)
MCA: Middle cerebral artery; CPR: Cerebroplacental Ratio
The finding of an abnormal Doppler parameter at the final scan prior to delivery was evaluated as a predictor of adverse perinatal outcome. The perinatal outcome parameters assessed were gestational age at delivery, preterm delivery at <34 weeks gestation, mean birth weight, birth weight <5th centile for gestation, birth weight discordance of ≥ 18%, neonatal intensive care unit admission, and a composite measure of adverse perinatal outcomes as previously described.

The level of 18% birth weight discordance was chosen, as an analysis of perinatal outcomes in the ESPRiT cohort found this level of discordance to be predictive of adverse outcomes in both MC and DC twins. The analysis of Doppler data was performed following the publication of this primary outcome from the ESPRiT data set. Therefore, all subsequent data analyses performed used the 18% level of discordance when evaluating outcomes. The association between abnormal Doppler parameters and adverse perinatal outcome was analysed by chorionicity.

Tables 4.4 and 4.5 detail the correlation between abnormal UA Doppler parameters and adverse outcomes in MC and DC twins. In the DC cohort, an abnormal UA Doppler prior to delivery was associated with earlier delivery, lower mean birthweight, and a higher chance of birthweight below 5th centile for gestation. Neonates were more likely to require NICU admission, but it was not predictive of overall perinatal morbidity, as assessed by a composite of morbidity outcomes. Similarly, for MC twins an abnormal UA was associated with lower mean birthweight and a higher chance of birthweight being below 5th centile. In contrast to DC twins, there was a significant association in MC twins between abnormal UA Doppler velocimeties and significant birthweight discordance. In the presence of an abnormal UA Doppler in one or both fetuses, the risk of birthweight discordance of 18% or greater was 42% in contrast to 17% where both fetuses had a normal UA Doppler (p= 0.0005). This association was reflected in the observation that an abnormal UA Doppler in MC twins was significantly associated with adverse neonatal outcomes.
MCA PI <5\textsuperscript{th} centile, present in 35.4% and 37.6% of MC and DC twins respectively prior to delivery, was a predictor of adverse clinical outcomes in DC twins only (Table 4.6). In this cohort, it was associated with both lower birthweight and birthweight below 5\textsuperscript{th} centile. In contrast, in MC twins, low MCA PI was not associated with any indicators of adverse outcome (Table 4.7).

An abnormal CPR, defined as a ratio of MCA PI:UA PI <1, was a good predictor of adverse outcome in the DC cohort (Table 4.8). In this group, it was significantly associated with all adverse clinical outcomes except preterm delivery. An earlier gestational age at delivery and higher rates of birthweight discordance, low birthweight, and birthweight <5\textsuperscript{th} centile were all observed when the CPR was <1 prior to delivery. This translated into a greater than 2-fold increase in the rate of adverse neonatal outcome. CPR <1 did not predict adverse outcome in MC twins (Table 4.9).

Uterine artery Dopplers were obtained on a very small percentage of the ESPRiT cohort. An abnormal UtA Doppler, defined as PI >95\textsuperscript{th} centile, was not associated with adverse outcomes in DC twins. In the MC cohort, an abnormal UtA Doppler was associated with an increased likelihood of BW < 5\textsuperscript{th} centile for gestational age (Table 4.10, 4.11). The small proportion of pregnancies with satisfactory UtA Doppler acquisition makes it difficult to draw any definitive conclusions on the UtA Doppler data.
Table 4.4: Abnormal UA Doppler at last assessment before delivery and association with adverse clinical outcome: Dichorionic Twins

<table>
<thead>
<tr>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N^a</td>
</tr>
<tr>
<td>Any UA assessment</td>
<td>774</td>
</tr>
<tr>
<td>Normal UA</td>
<td>594</td>
</tr>
<tr>
<td>Abnormal UA</td>
<td>180</td>
</tr>
<tr>
<td>P-value</td>
<td>0.004</td>
</tr>
</tbody>
</table>

^aNumber of UA assessments in at least one twin of a pair
^bMean Gestational age at delivery in weeks
^cBirthweight Discordance of \(\geq 18\%\)
Table 4.5: Abnormal UA Doppler at last assessment before delivery and association with adverse clinical outcome: Monochorionic Twins

<table>
<thead>
<tr>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Any UA assessment</td>
<td>171</td>
</tr>
<tr>
<td>Normal UA</td>
<td>126</td>
</tr>
<tr>
<td>Abnormal UA</td>
<td>45</td>
</tr>
<tr>
<td>p-value</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of UA assessments in at least one twin of a pair
<sup>b</sup>Mean Gestational age at delivery in weeks
<sup>c</sup>Birthweight Discordance of ≥18%
Table 4.6: Abnormal MCA Doppler at last assessment before delivery and association with adverse clinical outcome:

Dichorionic Twins

<table>
<thead>
<tr>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>N&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Any MCA assessment</td>
<td>765</td>
</tr>
<tr>
<td>Normal MCA</td>
<td>424</td>
</tr>
<tr>
<td>Abnormal MCA</td>
<td>341</td>
</tr>
<tr>
<td>p-value</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of MCA assessments in at least one twin of a pair

<sup>b</sup>Mean Gestational age at delivery in weeks

<sup>c</sup>Birthweight Discordance of ≥18%
Table 4.7: Abnormal MCA Doppler at last assessment before delivery and association with adverse clinical outcome: Monochorionic Twins

<table>
<thead>
<tr>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>GA at Delivery&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Any MCA assessment</td>
<td>168</td>
</tr>
<tr>
<td>Normal MCA</td>
<td>95</td>
</tr>
<tr>
<td>Abnormal MCA</td>
<td>73</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of MCA assessments in at least one twin of a pair
<sup>b</sup> Mean Gestational age at delivery in weeks
<sup>c</sup> Birthweight Discordance of ≥18%
Table 4.8: Abnormal Cerebroplacental Ratio at last assessment before delivery and association with adverse clinical outcome: Dichorionic Twins

<table>
<thead>
<tr>
<th></th>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GA at Delivery&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Any CPR assessment</td>
<td>764</td>
<td>37.1</td>
</tr>
<tr>
<td>Normal CPR</td>
<td>726</td>
<td>37.1</td>
</tr>
<tr>
<td>Abnormal CPR</td>
<td>38</td>
<td>35.8</td>
</tr>
<tr>
<td>p-value</td>
<td>0.03</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of CPR assessments in at least one twin of a pair  
<sup>b</sup>Mean Gestational age at delivery in weeks  
<sup>c</sup>Birthweight Discordance of ≥18%
Table 4.9: Abnormal Cerebroplacental Ratio at last assessment before delivery and association with adverse clinical outcome: Monochorionic Twins

<table>
<thead>
<tr>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(^a)</td>
</tr>
<tr>
<td>Any CPR assessment</td>
<td>168</td>
</tr>
<tr>
<td>Normal CPR</td>
<td>160</td>
</tr>
<tr>
<td>Abnormal CPR</td>
<td>8</td>
</tr>
<tr>
<td>p-value</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\)Number of CPR assessments in at least one twin of a pair  
\(^b\)Mean Gestational age at delivery in weeks  
\(^c\)Birthweight Discordance of \(\geq 18\%\)
Table 4.10: Abnormal Uterine Artery (UtA) Doppler and association with adverse clinical outcome: Dichorionic Twins

<table>
<thead>
<tr>
<th></th>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N¹</td>
<td>GA at Delivery¹</td>
</tr>
<tr>
<td>Any UtA assessment</td>
<td>117</td>
<td>37.0</td>
</tr>
<tr>
<td>Normal UtA</td>
<td>105</td>
<td>37.0</td>
</tr>
<tr>
<td>Abnormal UtA</td>
<td>12</td>
<td>36.1</td>
</tr>
<tr>
<td>p-value</td>
<td>0.07</td>
<td>0.52</td>
</tr>
</tbody>
</table>

¹Number of twin pregnancies with UtA assessments performed
²Mean Gestational age at delivery in weeks
³Birthweight Discordance of ≥18%
Table 4.11: Abnormal Uterine Artery (UtA) Doppler and association with adverse clinical outcome: Monochorionic Twins

<table>
<thead>
<tr>
<th>Twin Pair Outcomes</th>
<th>Individual Twin Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Any UtA assessment</td>
<td>21</td>
</tr>
<tr>
<td>Normal UtA</td>
<td>19</td>
</tr>
<tr>
<td>Abnormal UtA</td>
<td>2</td>
</tr>
<tr>
<td>p-value</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of twin pregnancies with UtA assessments performed

<sup>b</sup>Mean Gestational age at delivery in weeks

<sup>c</sup>Birthweight Discordance of ≥18%
Evaluating the correlation between placental pathological examination findings and antenatal sonographic assessment of multi-vessel Dopplers, the presence of a non-central insertion of the umbilical cord (marginal or velamentous) was not reflected in abnormalities of any of the Doppler parameters studied (Table 4.12).

The presence of placental histopathological lesions, as previously defined, was more common when the UA Doppler was abnormal in the DC cohort. A similar association was not found in MC twins. No other associations were found between placental abnormalities and Doppler studies.
Table 4.12: Correlation between abnormal antenatal Doppler findings and placental pathology

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Abnormal</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Central Cord Insertion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dichorionic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>17% (186)</td>
<td>15% (24)</td>
<td>0.4151</td>
</tr>
<tr>
<td>MCA</td>
<td>16% (120)</td>
<td>19% (83)</td>
<td>0.1541</td>
</tr>
<tr>
<td>CPR</td>
<td>17% (198)</td>
<td>9% (4)</td>
<td>0.1509</td>
</tr>
<tr>
<td>UtA</td>
<td>15% (26)</td>
<td>17% (4)</td>
<td>0.7899</td>
</tr>
<tr>
<td><strong>Monochorionic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>29% (66)</td>
<td>38% (18)</td>
<td>0.1984</td>
</tr>
<tr>
<td>MCA</td>
<td>28% (46)</td>
<td>36% (36)</td>
<td>0.1322</td>
</tr>
<tr>
<td>CPR</td>
<td>31% (80)</td>
<td>14% (1)</td>
<td>0.3433</td>
</tr>
<tr>
<td>UtA</td>
<td>29% (10)</td>
<td>25% (1)</td>
<td>0.8540</td>
</tr>
<tr>
<td><strong>Placental Histological Abnormalities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dichorionic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>32% (279)</td>
<td>41% (58)</td>
<td>0.0327</td>
</tr>
<tr>
<td>MCA</td>
<td>33% (203)</td>
<td>31% (115)</td>
<td>0.6036</td>
</tr>
<tr>
<td>CPR</td>
<td>32% (304)</td>
<td>28% (11)</td>
<td>0.5152</td>
</tr>
<tr>
<td>UtA</td>
<td>32% (44)</td>
<td>40% (8)</td>
<td>0.4570</td>
</tr>
<tr>
<td><strong>Monochorionic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>43% (89)</td>
<td>40% (17)</td>
<td>0.6401</td>
</tr>
<tr>
<td>MCA</td>
<td>42% (64)</td>
<td>48% (42)</td>
<td>0.3980</td>
</tr>
<tr>
<td>CPR</td>
<td>44% (103)</td>
<td>43% (3)</td>
<td>0.9435</td>
</tr>
<tr>
<td>UtA</td>
<td>38% (12)</td>
<td>50% (1)</td>
<td>0.7241</td>
</tr>
</tbody>
</table>

*Data are expressed as % (N)*

UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio; UtA: Uterine Artery
4.5 Discussion

It is now well established that twin pregnancies with evidence of growth discordance are at increased risk for perinatal mortality and morbidity.\(^{(73)}\) Placental histological examination is vital in the evaluation of intrauterine growth restriction. Studies in singletons have shown morphological changes in villous structure in placentas of fetuses affected by intrauterine growth restriction \((74, 86, 87)\). These abnormalities in villous development are a consequence of defective trophoblast invasion and impaired development of the uteroplacental circulation. Uteroplacental vascular disease has also been associated with placental infarcts in singleton pregnancies complicated by intrauterine growth restriction \((88)\).

Previous studies in twin pregnancies have evaluated placental disease as a contributor to birth weight discordance \((89-91)\). Most of the studies to date have concentrated on the monochorionic placenta, evaluating the abnormal vascular relations at play in the pathogenesis of twin-to-twin transfusion syndrome \((92-94)\). Several authors have also evaluated umbilical cord abnormalities and placental weight in the occurrence of discordant growth in twins \((95-97)\). However, there is a paucity of data in the literature on the gross and histological placental findings in monochorionic and dichorionic twins.

Studies published previously evaluating similar outcomes have been limited by relatively small numbers.\(^{(90, 91)}\) Inconsistencies in findings in these previous studies may be related to their size and their retrospective nature. In our study, the protocol for placental evaluation was established prior to commencing the study, and distinct pathological lesions were identified as potential aetiological factors in abnormal growth of twin pregnancies, thus minimizing variations in reporting of placental pathology outcomes.
In a prospectively assessed cohort of 668 twin pregnancies, we identified associations between placental pathological lesions and suboptimal growth. In dichorionic twins, we found that both discordant birth weight and small for gestational age status were significantly associated with underlying abnormalities in placental histological findings. Monochorionic twin pregnancies did not show a similar association.

As expected, we found that babies that had a birth weight less than the 5th centile for gestation had significantly higher rates of placental abnormalities than appropriately grown controls. When stratified by chorionicity, this finding held true only within the dichorionic cohort. However, there were only 16 SGA babies in the monochorionic cohort and therefore it is likely that the study was underpowered to detect a significant difference in this group.

Two previous publications in the literature assessed similar outcomes. A retrospective analysis of 147 twin placentas by Eberle et al (90) evaluated various placental pathological lesions, and found that discordant birth weight in dichorionic twins was related to increased numbers of placental lesions in lighter twins of discordant pairs. In a similar finding to our study, this association was not present in their monochorionic twin cohort. This study was limited, however, by very small numbers, with only 48 monochorionic twin pairs included.

A study by Victoria et al (91) included 388 dichorionic and 89 monochorionic twin pregnancies. A retrospective analysis of the placental pathology charts in this cohort showed significantly more vascular thrombotic lesions in the placental domains of smaller twins in discordant monochorionic pairs. However a similar association was not seen in the placentas of discordant dichorionic twins.
The previous chapter evaluates the role of cord insertion site and its relation to both birth weight discordance and small for gestational age status (95). The contrast in findings of these two arms of this study adds significantly to our knowledge of the differing pathological processes at play in monochorionic and dichorionic twin pregnancies complicated by growth abnormalities.

In the results set out in the previous chapter we established a significant association between non-central placental cord insertion sites and growth abnormalities in monochorionic twins. A similar association was not seen for dichorionic twins. In direct contrast, the results presented in this chapter suggest that for dichorionic twins, growth discordance may be related to underlying uteroplacental insufficiency selectively affecting one twin.

While there was a high frequency of placental abnormalities observed in monochorionic twins, this did not appear to influence the occurrence of birth weight discordance. This finding suggests that any placental lesions in monochorionic twin pregnancies are evenly distributed across the placental disc. The occurrence of significant growth discordance in these pregnancies is likely a result of an unequal distribution of placental mass between the two fetuses, abnormal cord insertion sites, or aberrant vascular connections.

Our study has a number of limitations. The number of placentas that were unavailable for examination represented one third of the entire study cohort. However, we believe that the 668 twin pregnancies with pathological data available were representative of the overall study group. Complicated twin pregnancies do not appear to be overrepresented in this dataset, as the proportion of monochorionic and dichorionic twins was equal to that in the overall cohort. Of the total number of twin pregnancies recruited to ESPRiT, 17.2% had >20% birth weight discordance. This is similar to the rate of 17.1% in this subgroup. The composite perinatal morbidity outcome
occurred in 17.4% of this group, not significantly different to the rate of 18.2% in the overall cohort (p=0.6). There were also no significant differences in gestational age at delivery, mean birth weight, or proportion of twins that were small for gestational age between the two groups.

Another potential limitation is the fact that placental examination was carried out in the delivery hospital rather than in a single pathology laboratory. To minimize differences in placental reporting, a detailed protocol for placental examination was designed, the pathological lesions to be assessed were pre-specified, and a standardized data collection sheet was used. Other placental pathology findings including signs of inflammation and fetal vascular lesions were inconsistently reported between centres, thus the analysis was limited to the lesions pre-specified in the study protocol.

Multi-vessel Doppler evaluation has been shown to have an important role in the evaluation and surveillance of at-risk pregnancies. The majority of published data linking Doppler abnormalities to clinical outcome relates to singleton pregnancies. Twin pregnancies have also been the focus of extensive research in this area. It is well established that serial ultrasound assessment of MC twin pregnancies, including Doppler assessment, is an integral part of surveillance for TTTS (98). Although, it is not routinely recommended in uncomplicated DC twin pregnancies, it has become common in clinical practice to include some degree of Doppler surveillance in management of these pregnancies.

It is therefore important to understand the frequency with which abnormal Doppler findings occur in twin pregnancies, when using singleton reference ranges, and to evaluate the clinical significance of such findings. Within the ESPRiT study design, we were able to firstly evaluate the overall occurrence of Doppler abnormalities in twins, and secondly to correlate these findings with clinical outcomes and placental examination findings.
Within our unselected cohort of structurally and chromosomally normal twin pregnancies, we found very high rates of Doppler abnormalities in both MC and DC twin pairs. Over one third of fetuses were found to have an abnormal UA Doppler on at least one occasion during the pregnancy. It was interesting to note that this did not differ between monochorionic and dichorionic twins. The finding that abnormal UA Doppler indices are associated with adverse perinatal outcomes in both MC and DC twins supports the use of this parameter in routine antenatal surveillance.

The very high rates of abnormal MCA PI in both MC and DC twins would suggest that the reference ranges for this Doppler may be different in twins from singleton pregnancies. One must therefore be cautious when interpreting an abnormal value and making clinical decisions on the basis of this. Interestingly, abnormal MCA Dopplers and an abnormal CPR were associated with adverse perinatal outcome in DC twins, but not in MC twins.

An abnormal CPR has been shown to be a good predictor of perinatal morbidity in growth restricted singletons and has been incorporated into the surveillance of complicated singleton pregnancies (99-101). Previous studies in twins have shown conflicting results to ours. A study from Gaziano et al (102) evaluated cerebral redistribution in 33 MC twin pairs and 50 DC twin pairs, and found that it occurred significantly more frequently in MC twins. They also noted an association between cerebral distribution and SGA in both MC and DC twins. Another small study of 23 MC and 52 DC twin pairs demonstrated an association between abnormal CPR and adverse perinatal outcome irrespective of chorionicity (103).

In contrast, we have shown that the overall rate of abnormal CPR did not differ between MC and DC twins, but it was only in the DC cohort that this abnormal Doppler parameter was predictive of adverse perinatal outcome. This most likely reflects that fact that, in DC twins, the pathophysiology
underscoring growth abnormalities is similar to that seen in singleton pregnancies. In contrast, MC twins develop aberrant growth patterns due to unequal placental sharing and abnormal placental vascular communications. It may be that these pathological mechanisms are not represented with the same pattern of ‘brain-sparing’ in Doppler studies.

These results suggest that interpretation of abnormal MC Doppler indices and CPR calculations must be approached with caution in MC twins and further research is needed to determine MC twin specific reference ranges for these parameters and to further correlate these with clinical outcomes.

The majority of published studies evaluating placental pathology and Doppler studies in twin pregnancies are restricted to the study of monochorionic twins, evaluating the role of vascular communications within the placenta.

In this study, we set out to assess the more common placental abnormalities and to evaluate their impact on antenatal Doppler studies. Although, as previously discussed, we have shown that marginal and velamentous placental cord insertion is associated with growth restriction in MC twins, this finding did not translate into any association between non-central cord insertion and antenatal Doppler abnormalities. Despite a trend towards increased rates of UA Doppler abnormalities with non-central cord insertion in MC twins, this did not reach statistical significance.

In contrast, our data suggest that placental histological abnormalities would be reflected in antenatal Doppler studies in DC, but not in MC twins. DC twins with placental features associated with uteroplacental insufficiency were significantly more likely to have an abnormal UA Doppler finding during antepartum surveillance. Once again, this is further evidence that the
underlying mechanism of growth discordance in DC twins is uteroplacental insufficiency, as seen in singleton IUGR pregnancies.

Although uteroplacental insufficiency is traditionally thought to reflect an abnormality in maternal uterine perfusion, this association with growth discordance in DC twins suggests that it may also have a fetal origin, as it can affect one twin and not the other. In order to further investigate this finding we selected a subgroup of DC twin placentas for stereological studies, the results of which are presented in the next chapter.

In conclusion, in a large, prospectively assessed cohort of monochorionic and dichorionic twins we have established an association between placental pathology and growth abnormalities. Together with the data relating cord insertion site to abnormal growth in twins, it adds further depth to our knowledge of the various pathological processes governing aberrant growth in twin pregnancies.

With respect to Doppler studies in twins, our data supports the routine use of UA Doppler assessment in both MC and DC twins. While MCA and CPR evaluation have a role in surveillance of complicated twin pregnancies, further studies in large twin cohorts should be performed to establish twin-specific reference ranges for these parameters.
Chapter 5 Results: Stereology

5.1 Introduction

Stereology, which enables calculation of three-dimensional quantitative data from two-dimensional specimens, has become an important tool in the study of the placenta. It has been used to evaluate placental morphology in normal pregnancy and in pregnancies complicated by a wide variety of conditions, including growth restriction, pre-eclampsia, diabetes, maternal anemia, and placenta praevia (38, 40, 104-107). To date, it has not been widely used in the study of twin placentas.

There are many factors influencing fetal growth during pregnancy, but normal placental development and functioning is crucial to support adequate intra-uterine growth. An important factor in placental development is maternal uterine perfusion, and studies have shown that abnormal uterine perfusion, as evidenced by abnormal uterine artery Doppler parameters, is associated with fetal growth restriction.

In dichorionic twin pregnancies, the uterine vasculature may adapt to support the perfusion of two placental masses, or there may be a change in placental architecture that enables fetal growth to proceed at a normal rate. Comparing stereological measurements of placental morphology between singleton and twin pregnancies with confirmed normal growth would advance our knowledge of the underlying processes governing growth in twin pregnancies.

Uteroplacental insufficiency is thought to result from maternal vascular malperfusion (MVM) (108); as stated above, abnormal uterine artery Dopplers have been shown to correlate with fetal growth restriction. More recently the term “ischaemic placental disease” has been used to refer to the
occurrence of intrauterine growth restriction (IUGR), pre-eclampsia, or placental abruption (109). It is thought that there is a common pathophysiological process underlying these conditions, with failure of adequate remodeling of maternal spiral arteries leading to defective trophoblast invasion (110). Several theories exist regarding the causes of defective spiral artery adaptation, including abnormalities in immunological factors, cytokines, and angiogenic signaling (111-114). However, not all placentas from pregnancies complicated by these conditions will display the classic features associated with MVM. A study by Walker et al examined 262 singleton placentas from pregnancies complicated by PET: only 54% showed evidence of impaired placental development with 60% displaying impaired villous development (115). A variety of other pathological lesions were found in the cohort including infarction (64%), haemorrhagic lesions (21%) and fetal vascular pathology (14%). A comprehensive histological examination is therefore vitally important to evaluate all potential placental causes in cases of PET or IUGR.

In a dichorionic twin pregnancy, it would be expected therefore that both placentas would have the same risk of placental ischaemic disease, as any maternal factors should equally apply to both placentas. We have shown in the previous chapter that selective IUGR affecting one twin of a dichorionic twin pair can be associated with similar placental histological features as seen in growth restricted singletons. This suggests that there are also fetal factors that influence the occurrence of uteroplacental insufficiency. In addition, the local uterine environment may influence placental development as evidenced by the fact that the upper twin is more frequently the smaller of the pair.

Stereological assessment of placentas from growth discordant dichorionic twins would allow further investigation of this phenomenon. The normally grown co-twin provides the ideal comparison for the growth-restricted twin to
evaluate the placental morphological abnormalities associated with growth restriction.

In addition, comparison with gestational age matched singletons from pregnancies complicated by maternal pre-eclampsia and IUGR may enable us to determine if the underlying placental abnormalities are similar in growth restricted twins.

5.2 Aim

The aim of this arm of the study was threefold:

1. To evaluate the adaptations in placental morphology that enable normal growth in twin pregnancies when compared to singleton placentas.
2. To investigate the hypothesis that uteroplacental insufficiency can affect one twin of a dichorionic twin pair.
3. To confirm that similar morphological abnormalities are present in placentas from growth-restricted twins as are seen in placentas from singletons with pre-eclampsia and IUGR.
5.3 Methods

As discussed in detail in chapter 2, placentas from dichorionic twin pregnancies enrolled in the ESPRiT study were selected for stereological analysis on the basis of inter-twin birthweight discordance, the presence or absence of accelerated villous maturation in the smaller twin, and the presence or absence of abnormal sonographic findings in the smaller twin. A group of twin placentas without birthweight discordance, and a group of normal and growth restricted singleton placentas were assessed as control groups.

Placentas included in this analysis were transferred to the Rotunda hospital, where they were sampled using a uniform random sampling method. Following sample preparation, stereological analysis was performed on 5 slides from each placenta, each of which contained 2 tissue sections. The volume and surface area of terminal villi and placental capillaries were then estimated, with the stereologically obtained values multiplied by placental volume to estimate total placental terminal villi and capillary volume. A single observer, blinded to clinical outcomes, performed the stereological analyses.

Stereological findings were then compared between clinically relevant subgroups, and the ability of antenatal sonography to predict stereological findings was assessed.

The candidate conceived and designed this study in conjunction with Dr. John Gillan, consultant pathologist, Rotunda hospital, and arranged transfer of the relevant placentas to the Rotunda where required. Stereological analysis was performed by an MSc student in Dr. Gillan’s laboratory, under the supervision of the candidate and Dr. Gillan. Data analysis was carried out by the candidate.
5.4 Results

5.4.1 Study Cohort

Stereological examination was performed on placentas of different cohorts of twin pairs, selected on the basis of inter-twin birthweight difference, placental histological findings, and results of antenatal ultrasound assessment. Placentas from singleton pregnancies provided a further 2 control groups. The initial study cohorts for stereological examination, as set out in the Material and Methods chapter, were subsequently refined to ensure that meaningful comparisons could be made between groups.

The twin cohorts were selected on the basis of percentage inter-twin birth weight discordance, defined as the difference in birthweight as a percentage of the birthweight of the larger twin, placental histological features, and antenatal ultrasound findings. Placental histological findings were reviewed to identify placentas with and without evidence of maternal vascular malperfusion (MVM). MVM on placental histology was considered evidence of pathological growth restriction.

Antenatal ultrasound reports were reviewed to identify a cohort of twins with sonographic features consistent with uteroplacental insufficiency. This was initially defined as either an abnormal UA Doppler - defined as absent end diastolic flow (AEDF) or reversed end diastolic flow (REDF) - or oligohydramnios. We subsequently limited the analysis to those with UA Doppler abnormalities only, as these were more truly representative of uteroplacental disease. It was not possible to definitively exclude other causes for oligohydramnios such as ruptured membranes; therefore, inclusion of these cases may have affected the results.
All cases were matched for gestational age and a further two, gestational age matched, singleton control groups were identified: uncomplicated singleton pregnancies and singleton pregnancies complicated by IUGR and preeclampsia.

Table 5.1 outlines the characteristics of the study group
Table 5.1: Study Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Title</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Singletons</td>
<td>10</td>
<td>Gestational age-matched singleton pregnancies with normal growth and normal placental histology</td>
</tr>
<tr>
<td>2</td>
<td>IUGR singletons</td>
<td>10</td>
<td>Gestational age-matched singleton pregnancies with PET/IUGR and confirmed MVM on placental histology</td>
</tr>
<tr>
<td>3</td>
<td>Normal Twins</td>
<td>10</td>
<td>Gestational age-matched twin pregnancies with normal growth and normal placental histology</td>
</tr>
<tr>
<td>4</td>
<td>“Non-pathological” growth discordant Twins</td>
<td>7</td>
<td>Twin pregnancies with &gt; 20% BW discordance, normal antenatal sonographic findings and normal placental histology</td>
</tr>
<tr>
<td></td>
<td>4A: AGA Twin; 4B: SGA Twin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>“Pathological” growth discordant Twins</td>
<td>6</td>
<td>Twin pregnancies with &gt; 20% BW discordance, smaller twin with AEDF/REDF on antenatal ultrasound and confirmed MVM on placental histology; larger twin with normal ultrasound and placental histology</td>
</tr>
<tr>
<td></td>
<td>5A: AGA Twin; 5B: SGA Twin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IUGR: Intrauterine growth restriction; PET: Preeclampsia; MVM: maternal vascular malperfusion; BW: Birth weight; AGA: Appropriate for gestational age; SGA: Small for gestational age
The stereological outputs obtained for surface area and volume measurements of terminal villi and capillaries were multiplied by the placental volume for each case. This gave values for total surface area and total volume.

The mean values for each group were compared across all 5 groups and then the following specific comparisons were performed:

1. Normal twins and normal singletons
2. AGA and SGA twins in “non-pathological” growth discordant twins
3. AGA and SGA twins in “pathological” growth discordant twins
4. “Non-pathological” SGA twins and “pathological” SGA twins
5. SGA twins and IUGR singleton

5.4.2 Overall Data

In total, stereological assessment was carried out on 20 singleton placentas and 46 twin placentas (23 pairs) as per the grouping described above. The distribution of birth weights across the groups is depicted in Figure 5.1. As expected, given the cohort selection, there were differences in birth weight noted across the various groups and between the twin pairs in Groups 4 and 5. Of note, the mean birth weight of the normal singletons was similar to that of the normally grown twin group.
Figure 5.1 Birthweights

Figures 5.2 to 5.5 show the distribution of the 4 placental stereological parameters assessed across the study groups. The data presented represent the mean measurements for each group.

Figure 5.2 Terminal Villi: Total Surface Area
Figure 5.3 Terminal Villi: Total Volume

Figure 5.4 Capillaries: Total Surface Area
In order to investigate the three specific aims of the study, as detailed above, select comparisons were performed from the appropriate study groups. These results are presented in the next section.
5.4.3 Normal twins compared to normal singletons

Placental architecture in 20 twins with normal growth, normal placental histopathology, and no antenatal sonographic evidence of oligohydramnios or UA Doppler abnormalities was compared to 10 gestational-age matched singletons. (Table 5.2)

There were no differences in the terminal villi in either total surface area or total volume.

However, capillary development differed significantly in twin placentas, with the total surface area of the capillaries in twin placentas being significantly greater that that in singleton placentas (20.53m² v 15.55m², p=0.02). In addition, there was a greater total capillary volume in twin placentas, although not to a statistically significant degree (82.93cm³ v 62.50cm³, p=0.09).
Table 5.2: Comparison of stereological variables in normal twins and normal singletons

<table>
<thead>
<tr>
<th></th>
<th>Mean (SEM)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td><strong>Terminal Villi: Total Surface</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>14.90m²(0.62)</td>
<td>0.98</td>
</tr>
<tr>
<td>AGA Singleton</td>
<td>14.87m²(1.15)</td>
<td></td>
</tr>
<tr>
<td><strong>Terminal Villi: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>215.05cm³(15.35)</td>
<td>0.89</td>
</tr>
<tr>
<td>AGA Singleton</td>
<td>211.88cm³(16.31)</td>
<td></td>
</tr>
<tr>
<td><strong>Capillaries: Total Surface</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>20.53m²(1.13)</td>
<td>0.02</td>
</tr>
<tr>
<td>AGA Singleton</td>
<td>15.55m²(1.70)</td>
<td></td>
</tr>
<tr>
<td><strong>Capillaries: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>82.93cm³(7.10)</td>
<td>0.09</td>
</tr>
<tr>
<td>AGA Singleton</td>
<td>62.50cm³(8.41)</td>
<td></td>
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</tbody>
</table>

SEM; Standard error of the mean; AGA: appropriate for gestational age; SGA: small for gestational age.
5.4.4 “Non-pathological” growth discordant twins: AGA twin compared to SGA twin

Within the cohort of 7 DC twin pregnancies with evidence of growth discordance, but no features suggestive of a pathological basis for the growth discordance, significant differences were found in the placental structure of AGA and SGA twins. *(table 5.3)*

Stereological measurements of placentas from SGA twins showed significantly smaller terminal villi, both in terms of surface area (17.43m$^2$ v 13.39m$^2$, p=0.03) and volume (265.00cm$^3$ v 200.31cm$^3$, p=0.04).

Capillary architecture was also different, with reduced capillary surface area (23.74m$^2$ v 18.69m$^2$, p=0.03) and a non-statistically significant reduction in total capillary volume in the placentas of SGA twins when compared to their appropriately grown co-twins.
Table 5.3: ‘Non-pathological’ growth discordant twins; comparison of stereological variables

<table>
<thead>
<tr>
<th></th>
<th>Mean (SEM)</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>Terminal Villi: Total Surface Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>17.43m²(1.19)</td>
<td>0.03</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>13.39m²(1.16)</td>
<td></td>
</tr>
<tr>
<td><strong>Terminal Villi: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>265.00cm³(18.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>200.31cm³(21.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Capillaries: Total Surface Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>23.74m²(1.67)</td>
<td>0.03</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>18.69m²(1.34)</td>
<td></td>
</tr>
<tr>
<td><strong>Capillaries: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>100.5cm³(13.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>76.9cm³(6.6)</td>
<td></td>
</tr>
</tbody>
</table>

SEM; Standard error of the mean; AGA: appropriate for gestational age; SGA: small for gestational age.
5.4.5 “Pathological” growth discordant twins: AGA twin compared to SGA twin

A within-group comparison was then performed on the cohort of 6 twin pregnancies with pathological growth restriction affecting one twin of the pair, as evidenced by an abnormal UA Doppler prior to delivery and confirmed MVM at placental histological examination. All 6 growth restricted twins had AEDF in the UA prior to delivery. There were no cases of REDF included. The appropriately grown co-twins had normal Doppler sonography throughout pregnancy and placental examination demonstrated normal histological features.

Stark differences were found when these placentas underwent stereological quantification: (table 5.4) the placentas of growth restricted twins demonstrated an approximate 50% reduction in terminal villous surface area and volume and capillary surface area and volume.
Table 5.4: ‘Pathological’ growth discordant twins; comparison of stereological variables

<table>
<thead>
<tr>
<th></th>
<th>Mean (SEM)</th>
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<tr>
<td><strong>Terminal Villi:</strong></td>
<td></td>
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<tr>
<td>Total Surface Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>14.67m²(0.47)</td>
<td>&lt;0.0001</td>
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<tr>
<td>SGA Twin</td>
<td>8.69m²(1.78)</td>
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</tr>
<tr>
<td>Total Volume</td>
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</tr>
<tr>
<td>AGA Twin</td>
<td>206.7cm³(9.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>122.3cm³(21.1)</td>
<td></td>
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<tr>
<td><strong>Capillaries:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Surface Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>21.41m²(0.96)</td>
<td>0.0002</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>10.49m²(1.62)</td>
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<tr>
<td>Total Volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA Twin</td>
<td>90.63cm³(6.03)</td>
<td>0.0005</td>
</tr>
<tr>
<td>SGA Twin</td>
<td>42.05cm³(7.36)</td>
<td></td>
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</table>

*SEM: Standard error of the mean; AGA: appropriate for gestational age; SGA: small for gestational age.*
5.4.6 SGA twins: Comparison of growth restricted twins with normal UA Dopplers and growth restricted twins with AEDF in UA

A between group comparison was performed on the cohort of 7 SGA twins with normal antenatal Doppler velocimetries and the cohort of 6 growth restricted twins in whom antenatal sonography demonstrated AEDF in the UA.

The mean values for the 4 placental stereological variables assessed were compared between these two groups (table 5.5), with significant reductions found in all measures of placental villous and capillary growth when the UA Doppler demonstrated AEDF. There was a 35% reduction in the mean surface area of the terminal villi and a 40% reduction in total volume of terminal villi. With respect to capillary parameter, surface area and volume were reduced by almost 50% in the placentas of the abnormal Doppler group when compared to those with normal UA Doppler studies.
Table 5.5: SGA twins: Comparison of stereological variables in twins with normal and abnormal UA Doppler studies

<table>
<thead>
<tr>
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<th>Mean (SEM)</th>
<th>p-value</th>
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<tr>
<td><strong>Terminal Villi: Total Surface</strong></td>
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<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal UA Doppler</td>
<td>13.38m²(1.16)</td>
<td>0.007</td>
</tr>
<tr>
<td>UA Doppler: AEDF</td>
<td>8.69m²(0.73)</td>
<td></td>
</tr>
<tr>
<td><strong>Terminal Villi: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal UA Doppler</td>
<td>200.3cm³(21.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>UA Doppler: AEDF</td>
<td>122.3cm³(21.1)</td>
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<td><strong>Capillaries: Total Surface</strong></td>
<td></td>
<td></td>
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<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal UA Doppler</td>
<td>18.69m²(1.34)</td>
<td>0.002</td>
</tr>
<tr>
<td>UA Doppler: AEDF</td>
<td>10.49m²(1.62)</td>
<td></td>
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<tr>
<td><strong>Capillaries: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal UA Doppler</td>
<td>76.9cm³(6.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>UA Doppler: AEDF</td>
<td>42.05cm³(7.36)</td>
<td></td>
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</tbody>
</table>

*SEM: Standard error of the mean; AEDF: Absent end-diastolic flow*
5.4.7 IUGR: Comparison of placental morphology in twin and singleton pregnancies complicated by IUGR

Finally, a three-way comparison was performed to compare twin pregnancies and singleton pregnancies complicated by fetal growth restriction. (Table 5.6) A cohort of 10 singleton pregnancies with maternal pre-eclampsia and fetal growth restriction was used as the comparison group. These were initially compared to the 7, apparently non-pathological, IUGR twins and, secondly, to the growth restricted twins with abnormal antenatal Doppler sonography. As with all previous comparisons, the groups were matched for gestational age.

Within the initial comparison group of IUGR singletons and IUGR twins with normal UA Doppler studies, no differences were observed in placental villous measurements. However, capillary growth was significantly increased in the SGA twin placenta when compared to the IUGR singletons.

Conversely, similar patterns of placental remodeling were found in pathologically growth restricted twins and IUGR singletons. There was a similar reduction in villous volume, capillary surface area and capillary volume in these two groups, although a significantly greater reduction in terminal villous surface area was seen in the SGA twins compared to the SGA singletons.
Table 5.6: Stereological variables in growth restricted singletons and growth restricted twins, with and without abnormal UA Doppler studies

<table>
<thead>
<tr>
<th></th>
<th>Mean (SEM)</th>
<th>p-value*</th>
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<tr>
<td><strong>Terminal Villi: Total Surface</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUGR Singleton</td>
<td>12.45m²(1.17)</td>
<td></td>
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<tr>
<td>SGA Twin; normal UA</td>
<td>13.38m²(1.16)</td>
<td>0.59</td>
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<tr>
<td>Doppler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA Twin; abnormal UA</td>
<td>8.69m²(0.73)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Terminal Villi: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUGR Singleton</td>
<td>156.26m³(15.6)</td>
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</tr>
<tr>
<td>SGA Twin; normal UA</td>
<td>200.3cm³(21.7)</td>
<td>0.11</td>
</tr>
<tr>
<td>Doppler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA Twin; abnormal UA</td>
<td>122.3cm³(21.1)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Capillaries: Total Surface</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUGR Singleton</td>
<td>13.48m²(1.23)</td>
<td></td>
</tr>
<tr>
<td>SGA Twin; normal UA</td>
<td>18.69m²(1.34)</td>
<td>0.01</td>
</tr>
<tr>
<td>Doppler</td>
<td></td>
<td></td>
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<tr>
<td>SGA Twin; abnormal UA</td>
<td>10.49m²(1.62)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Capillaries: Total Volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUGR Singleton</td>
<td>44.26m³(3.1)</td>
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<td>76.9cm³(6.6)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Doppler</td>
<td></td>
<td></td>
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<tr>
<td>SGA Twin; abnormal UA</td>
<td>42.05cm³(7.36)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*Comparison with IUGR singleton

SEM: standard error of the mean; IUGR: Intrauterine growth restriction; SGA: Small for gestational age
5.4 Discussion

Stereology has become a valuable investigative tool in the study of placental morphology, enabling accurate, non-biased measurement of placental structure. To date, its use has been confined to the study of singleton placentas, with no prior published studies using stereology to study twin placentas. Consequently, these data allow novel insights into placental adaptation in twin pregnancies, as well as providing further knowledge of the pathophysiological processes underlying growth abnormalities in dichorionic twin pregnancies.

Fetal growth in multiple gestations is governed by a myriad of factors. Normal placental development and adaptation is vital to ensuring normal growth in all pregnancies. It is well recognised that fetuses in twin gestations grow at a different rate to singletons. Beyond 24 weeks gestations, the rate of growth in twin pregnancies slows when compared to singletons (116), and when compared to twins, birthweight in triplets is lower, irrespective of chorionicity (117). This observation that increasing fetal number is linked to decreasing birth weight led to the term “placental crowding” to describe the phenomenon that with increasing fetal number the relative placental weights reduce (14). Further support for this “placental crowding” hypothesis comes from the observation that, in dichorionic twin pregnancies, placental proximity influences growth. Dichorionic twins with separate placental masses have been shown to have higher birth weights and a reduced chance of one or two SGA twins than those with fused a placental mass (15).

However, despite this “placental crowding”, the uterus and its vasculature is capable of supporting satisfactory fetal growth in multiple gestations. This observation raises the question of what placental adaptations are necessary in twin pregnancies to enable the normal growth of two fetuses. In order to address this question, we compared the stereological outputs from the placentas of normally grown twins with those of gestational age matched, appropriately grown singletons.
There was no significant difference in the birth weights in these 2 groups (2463g vs. 2511g; \( p=0.94 \)). Our results showed significant differences in the placental morphology of twins and singletons, and particularly in capillary morphology, with twin placentas demonstrating an increase in volume and surface area of capillaries.

This may be an adaptive mechanism to enable normal placental transfer of oxygen and nutrients. When faced with a fixed maternal blood supply, the placenta appears capable of increasing its vascular component to support two placentas competing for the uterine blood supply. This phenomenon has not been previously described but it has a credible biological basis and warrants further investigation.

The second aim of our study was to investigate the role of uteroplacental insufficiency in growth discordance in dichorionic twin pregnancies. Uteroplacental insufficiency is a well described phenomenon in the pathogenesis of IUGR (118). Stereology has been used to study placentas affected by uteroplacental insufficiency and fetal growth restriction in singletons (40, 104), but has not previously been utilized in the study of growth restricted twin pregnancies.

Maternal factors such as preeclampsia, high-altitude, and asthma have previously been shown to be associated with significant stereological abnormalities, particularly in the presence of fetal growth restriction (119, 120). However, in twin pregnancy the maternal influence is the same on both placental masses, so one would therefore expect that the placental morphology would be similar in the placentas of a twin pair. In practice, growth restriction can selectively affect one twin, even in a dichorionic twin pair. When structural and chromosomal abnormalities are excluded, much of this difference may be explained by differing genetic growth potential, but the observation that growth discordant dichorionic twins have increased rates of perinatal morbidity and mortality compared to concordantly grown dichorionic twins suggests that, in at least some cases, the presence of inter twin growth discordance is pathological.
We aimed to assess the potential contribution of placental morphology to the development of growth discordance in non-pathologically and pathologically growth discordant dichorionic twins, finding that placental morphology varied considerably between the smaller and larger twins of growth discordant pairs, even in the absence of sonographic markers of significant uteroplacental dysfunction.

As data in singletons have shown, IUGR is associated with a reduction in the placental diffusing capacity, as evidenced by reduced volume and surface area of both terminal villi and capillaries. A study by Todros et al, comparing placental villus architecture in growth restricted singletons showed marked differences in those with positive EDF in the UA compared to those with AREDF (121). AREDF was associated with significantly fewer gas-exchanging villi with poor capillary development. In our cohort, the evidence of reduced placental diffusing capacity was more marked in the placentas of twins in whom antenatal Doppler abnormalities were present, potentially justifying the routine use of UA Doppler surveillance in dichorionic twin pregnancies to screen for underlying placental pathology.

Finally, we compared placental morphology in growth restricted twins to growth restricted singletons from pregnancies complicated by preeclampsia, observing that the placentas from pathologically growth restricted twins had a similar reduction in terminal villous volume and capillary volume and surface area to the pre-eclamptic singleton placentas. In contrast, the smaller twins from the ‘non-pathological’ growth restricted twin pairs actually demonstrated increased capillary volume and surface area when compared to the IUGR singleton group.

This would appear to represent a similar adaptive mechanism to that seen in the normal twin pairs. It could therefore be that a failure of this adaptive mechanism underpins the development of ‘pathological’ growth restriction. The results of these studies suggest that uteroplacental insufficiency can affect one twin of a dichorionic twin pair, but not their co-twin. The
placental changes seen, with reduced placental and villous mass leading to a reduced capacity for placental transfer of oxygen and nutrients, are similar to those observed in placentas from pregnancies affected by preeclampsia. How these changes can occur in one placenta and not the other, both of which occupy the same uterus, demands further investigation. Potential contributory elements include underlying fetal genetic factors and the site of placental implantation. Different areas of the uterus may have relative differences in maternal blood supply, with placental implantation in a relative less well-perfused leading to subsequent IUGR.

These data add further to our knowledge of the adaptive mechanisms underlying placental growth and development. The use of stereological measurement of placental morphology allows the identification and quantification of terminal villous and vascular changes seen in normal and complicated dichorionic twin pregnancies. Further studies should aim to confirm these findings of placental adaptation in twin pregnancies and further explore the phenomenon of uteroplacental insufficiency selectively affecting one placenta of a twin pair.
Chapter 6: Conclusions and Recommendations for Future Research

Management of multiple gestations remains one of the biggest challenges in perinatal medicine. Although there has been a decrease in triplet and higher-order multiple pregnancies as single or double embryo transfer has become the standard of care in in-vitro fertilization cycles, the rate of twinning remains high. As women delay childbearing to ever-later ages this high rate of twin pregnancies is unlikely to reduce.

The risks of a twin pregnancy are multiple, with increased rates of maternal complications such as pre-eclampsia, hypertensive disorders, and gestational diabetes. It is, however, increased perinatal morbidity that contributes most to the economic burden of twin pregnancies. High rates of preterm delivery, both spontaneous and indicated, lead to high costs for prolonged neonatal intensive care stays and the long-term costs associated with the complications of preterm delivery, such as cerebral palsy, developmental delay, and blindness.

With these significant clinical and financial implications, twin pregnancies are worthy of ongoing research in order to optimize management, identify complications in a timely manner, and intervene appropriately. Growth aberrations contribute significantly to the excess perinatal morbidity and mortality associated with twin pregnancy; improving our knowledge of the underlying pathophysiological processes in growth discordant twin pregnancies is a clear research priority.

Since serial sonographic surveillance is the mainstay of antenatal management, identification of sonographic features associated with these underlying pathological processes should help better risk-stratification of twin pregnancies, and also facilitate the design of appropriate antenatal surveillance regimens. The findings presented in this thesis add to the body of published research in twins and will help to inform our management of these pregnancies.
Although there have been a multitude of studies published in the literature examining the basis of growth abnormalities and placental pathological features in twin pregnancies, the majority of these have focused on monochorionic twins, or failed to stratify their results by chorionicity. It seems likely that the pathological basis of abnormal growth differs by chorionicity, and our large prospectively recruited cohort of twins enabled us to accurately study these differences.

When evaluating macroscopic and microscopic placental pathological features, we found contrasting associations with growth restriction in monochorionic and dichorionic twins. The finding that an abnormal placental cord insertion is associated with abnormal growth in monochorionic twins supports the theory that differences in growth when a twin pair has a single placenta are due to an unequal sharing of the placental mass. We also know that abnormal vascular communications in the placenta contribute to differences in growth for monochorionic twins, and these abnormal vascular communications may be more common in those placentas with marginal or velamentous cord insertions. The use of advanced placental examination using dye-injection techniques to study vascular distribution and relative placental territories in the presence of normal and abnormal placental cord insertions would be an ideal way to further investigate this area.

Our finding that abnormal cord insertion is associated with suboptimal growth in monochorionic twins should prompt consideration of routinely evaluating cord insertion on antenatal ultrasound and modifying antenatal sonographic surveillance accordingly. However, further study is needed to evaluate the accuracy of antenatal ultrasound in determining placental cord insertion sites in twin pregnancies and to determine the optimal gestational at which this should be evaluated.

In contrast, the findings of our placental histopathological examinations, supported by the stereology findings, suggest that the underlying mechanism of growth restriction in dichorionic twins may be
uteroplacental insufficiency similar to that seen in growth-restricted singletons. This is supported by the finding that Doppler abnormalities associated with adverse perinatal outcome in dichorionic twins are similar to those seen in singletons. Abnormal MCA Doppler and CPR reflecting ‘brain-sparing’ - as blood is preferentially cerebrally distributed - are known to predict adverse perinatal outcome in singleton pregnancies; our data suggest that this is also the case in dichorionic twins. Measurement of these parameters should therefore be considered in surveillance of growth restricted dichorionic twins. Further study is recommended, however, to define normal values for MCA Dopplers in both monochorionic and dichorionic twins.

The findings of our stereology studies provide further insight into the adaptive mechanisms occurring in twin placentas to enable satisfactory growth. These data suggest that uteroplacental insufficiency can be associated with growth restriction in dichorionic twins, with similar placental morphological changes to that seen in growth restricted singletons. That this can occur in one placenta, and not the other, developing within one uterus is fascinating and certainly demands further investigation.

This is the first study using stereology as a tool to evaluate twin placentas and our findings support its use in further studies. In order to further progress the findings of this study, stereological analysis of dichorionic placentas, contrasting fused and unfused placental masses, should be considered in order to further investigate the placental crowding hypothesis.

In addition, stereological assessment of monochorionic placentas would allow the evaluation of adaptive differences from dichorionic placentas and from singletons. Similarly, stereological comparisons of villous and vascular morphology from each placental territory of monochorionic twins with growth discordance, abnormal placental cord insertions or placental
vascular anomalies would enhance our knowledge of the pathophysiology of growth restriction in such pregnancies.

In summary, our study of placental pathology in a large prospectively assessed cohort of monochorionic and dichorionic twin pregnancies has enhanced the understanding of the placental pathological processes which underscore normal and disorder growth of twins. This knowledge can be used to further refine our antenatal surveillance strategies, with the aim of improving perinatal outcome in this high-risk group.
Appendix 1

Supplementary Data
### Overall Study Cohort

#### Study Participants

<table>
<thead>
<tr>
<th>Recruited</th>
<th>Withdrew/Lost to follow up</th>
<th>Completed Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1028</td>
<td>27</td>
<td>1001</td>
</tr>
</tbody>
</table>

- Preivable demise of one or both fetuses: 24
- Perinatal Outcome data recorded: 977

#### Chorionicity

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichorionic</td>
<td>789</td>
</tr>
<tr>
<td>Monochorionic</td>
<td>188</td>
</tr>
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</table>

#### Maternal Characteristics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Mean Age (yrs)</td>
<td>32.6</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>503 (50.2%)</td>
</tr>
<tr>
<td>Assisted Conception</td>
<td>223 (23.3%)</td>
</tr>
<tr>
<td>Smoker</td>
<td>114 (11.4%)</td>
</tr>
<tr>
<td>Mean BMI</td>
<td>25.5</td>
</tr>
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#### Obstetric Complications

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>GH/PET</td>
<td>92 (9.2%)</td>
</tr>
<tr>
<td>GDM</td>
<td>14 (1.4%)</td>
</tr>
<tr>
<td>PPROM</td>
<td>26 (2.6%)</td>
</tr>
<tr>
<td>Placental Abruption</td>
<td>5 (0.5%)</td>
</tr>
<tr>
<td>Placenta Praevia</td>
<td>9 (0.9%)</td>
</tr>
<tr>
<td>TTTS</td>
<td>14 (7%)*</td>
</tr>
</tbody>
</table>

*Data expressed as n (% of total); *% of MC twins; GH: Gestational Hypertension; PET: Preeclampsia; PPROM: Preterm premature rupture of membranes; TTTS: Twin to twin transfusion syndrome*
Perinatal Outcomes by Chorionicity

<table>
<thead>
<tr>
<th></th>
<th>Monochorionic</th>
<th>Dichorionic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean GA at Delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(wks)</td>
<td>35.7</td>
<td>37.1</td>
</tr>
<tr>
<td><strong>Mean Birthweight (g)</strong></td>
<td>2335</td>
<td>2580</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscarriage</td>
<td>7 (3.5%)</td>
<td>9 (1.1%)</td>
</tr>
<tr>
<td>Single IUFD</td>
<td>11 (5.5%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Dual IUFD</td>
<td>2 (1%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>NND</td>
<td>1 (0.5%)</td>
<td>3 (0.4%)</td>
</tr>
<tr>
<td><strong>Morbidity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NICU admission</td>
<td>224 (60%)</td>
<td>655 (41%)</td>
</tr>
<tr>
<td>HIE</td>
<td>1 (0.3%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>PVL</td>
<td>3 (0.8%)</td>
<td>3 (0.2%)</td>
</tr>
<tr>
<td>NEC</td>
<td>4 (1%)</td>
<td>7 (0.4%)</td>
</tr>
<tr>
<td>RDS</td>
<td>85 (23%)</td>
<td>194 (12%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>41 (11%)</td>
<td>74 (5%)</td>
</tr>
<tr>
<td><strong>Composite Perinatal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morbidity</td>
<td>99 (26%)</td>
<td>213 (13%)</td>
</tr>
</tbody>
</table>

Data are expressed as n(%); GA: gestational age; IUFD: intrauterine fetal demise; NND: neonatal demise; NICU: neonatal intensive care unit; HIE: hypoxic ischaemic encephalopathy; PVL: periventricular leukomalacia; NEC: necrotizing enterocolitis; RDS: respiratory distress syndrome
Cord Insertion Data

**Overall Cohort (n = 816 twin pairs)**

<table>
<thead>
<tr>
<th>Type of Insertion</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central CI</td>
<td>1305</td>
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<tr>
<td>Marginal CI</td>
<td>250</td>
<td>15.3%</td>
</tr>
<tr>
<td>Velamentous CI</td>
<td>77</td>
<td>4.7%</td>
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</table>

**Monochorionic Twins (n = 165 twin pairs)**

<table>
<thead>
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<th>Type of Insertion</th>
<th>Count</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Central CI</td>
<td>226</td>
<td>68.5%</td>
</tr>
<tr>
<td>Marginal CI</td>
<td>73</td>
<td>22.1%</td>
</tr>
<tr>
<td>Velamentous CI</td>
<td>31</td>
<td>9.4%</td>
</tr>
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</table>

**Dichorionic Twins (n = 651 twin pairs)**

<table>
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<th>Type of Insertion</th>
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<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Central CI</td>
<td>1079</td>
<td>82.9%</td>
</tr>
<tr>
<td>Marginal CI</td>
<td>177</td>
<td>13.6%</td>
</tr>
<tr>
<td>Velamentous CI</td>
<td>46</td>
<td>3.5%</td>
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### Doppler Data

<table>
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<th>Assessments</th>
<th>Monochorionics</th>
<th>Dichorionics</th>
<th>All</th>
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<tr>
<td>Total UA PI assessments</td>
<td>2182</td>
<td>9740</td>
<td>11922</td>
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<tr>
<td>Total MCA PI assessments</td>
<td>1302</td>
<td>5982</td>
<td>7284</td>
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<tr>
<td>Total CPR assessments</td>
<td>1234</td>
<td>5860</td>
<td>7094</td>
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<td>Total UtA PI assessments</td>
<td>44</td>
<td>241</td>
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<table>
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<tr>
<th>Number of Fetus'</th>
<th>N=348</th>
<th>N=1556</th>
<th>N=1904</th>
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<tr>
<td>With at least 1 UA assessment</td>
<td>342</td>
<td>1548</td>
<td>1890</td>
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<td>With at least 1 MCA assessment</td>
<td>330</td>
<td>1480</td>
<td>1810</td>
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<td>With at least 1 CPR assessment</td>
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<td>1472</td>
<td>1800</td>
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<td>With at least 1 UtA assessment</td>
<td>42</td>
<td>233</td>
<td>275</td>
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<tr>
<td>Adverse composite outcome</td>
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<td>292</td>
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<tr>
<td>SGA (5th centile)</td>
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<td>Non-central cord insertion</td>
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<td>1534</td>
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<td>Placental abnormality</td>
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<td>445</td>
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<table>
<thead>
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<th>N=778</th>
<th>N=952</th>
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<tr>
<td>With at least 1 UA assessment</td>
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<td>774</td>
<td>945</td>
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<tr>
<td>With at least 1 MCA assessment</td>
<td>168</td>
<td>765</td>
<td>933</td>
</tr>
<tr>
<td>With at least 1 CPR assessment</td>
<td>168</td>
<td>764</td>
<td>932</td>
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<tr>
<td>With at least 1 UtA assessment</td>
<td>21</td>
<td>117</td>
<td>138</td>
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<tr>
<td>Pre-term (&lt;34 weeks)</td>
<td>44</td>
<td>97</td>
<td>141</td>
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<tr>
<td>BW discordance &gt; 18%</td>
<td>41</td>
<td>165</td>
<td>206</td>
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## Doppler and Outcome Data

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<th>Any Abnormal MCA</th>
<th>Any Abnormal CPR</th>
<th>Last examination</th>
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<tr>
<td><strong>Monochorionic Twins</strong></td>
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<td></td>
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<tr>
<td>GA at Delivery (mean)</td>
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<td>35.1</td>
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<td>2277</td>
<td>2284</td>
<td>2281</td>
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<tr>
<td>Adverse outcome</td>
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<td>41 (100%)</td>
<td>7 (18%)</td>
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<td>37 (90%)</td>
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<tr>
<td>BW &lt;5th Centile</td>
<td>18</td>
<td>17 (100%)</td>
<td>4 (24%)</td>
<td>3 (18%)</td>
<td>15 (88%)</td>
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<td>42 (100%)</td>
<td>6 (15%)</td>
<td>11 (27%)</td>
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<td>NICU admission</td>
<td>102</td>
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<td>22 (22%)</td>
<td>88 (89%)</td>
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<td>42</td>
<td>40 (100%)</td>
<td>8 (21%)</td>
<td>9 (24%)</td>
<td>39 (98%)</td>
</tr>
<tr>
<td>Non-central cord insertion</td>
<td>71</td>
<td>68 (100%)</td>
<td>10 (15%)</td>
<td>18 (27%)</td>
<td>63 (93%)</td>
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<tr>
<td><strong>Dichorionic Twins</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA at Delivery (mean)</td>
<td>36.3</td>
<td>36.4</td>
<td>36.4</td>
<td>36.2</td>
<td>36.3</td>
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<td>BW (mean)</td>
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<td>2516</td>
<td>2519</td>
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<td>2508</td>
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<td>15 (14%)</td>
<td>25 (23%)</td>
<td>98 (91%)</td>
</tr>
<tr>
<td>BW &lt;5th Centile</td>
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<td>100 (99%)</td>
<td>20 (20%)</td>
<td>38 (39%)</td>
<td>94 (93%)</td>
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<td>92 (96%)</td>
<td>14 (15%)</td>
<td>21 (23%)</td>
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<td>81 (23%)</td>
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<tr>
<td>BW discordance &gt;18%</td>
<td>165</td>
<td>160 (98%)</td>
<td>27 (17%)</td>
<td>37 (23%)</td>
<td>149 (91%)</td>
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<tr>
<td>Non-central cord insertion</td>
<td>173</td>
<td>162 (94%)</td>
<td>36 (21%)</td>
<td>29 (17%)</td>
<td>149 (86%)</td>
</tr>
</tbody>
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Appendix 2

Presentations and Publications
Presentations

Oral Presentations:

1. Abnormal cerebroplacental ratio predicts adverse outcomes in dichorionic twins
   British Maternal Fetal Medicine Society Meeting, Harrogate, 2014

2. Placental cord insertion and birthweight discordance in twin pregnancies: results of the national prospective ESPRiT trial.

Poster Presentations:

1. Perinatal Outcome in twins discordant for umbilical arterial Doppler abnormalities.
   Etaoin Kent, Fionnuala Breathnach, Gerry Burke, Fionnuala McAuliffe, Michael Geary, Sean Daly, John Higgins, Alyson Hunter, John Morrison, Shane Higgins, Rhona Mahony, Patrick Dicker, Fiona Manning, Elizabeth Tully, Fergal Malone
   Society for Maternal and Fetal Medicine Annual Meeting, San Diego, 2015

2. Relationship between placental characteristics and antenatal ultrasound Doppler indices in twin pregnancies – results of the ESPRiT Study.
   Etaoin Kent, Fionnuala Breathnach, John Gillan, Fionnuala McAuliffe, Michael Geary, Sean Daly, John Higgins, Alyson Hunter, John Morrison, Gerard Burke, Shane Higgins, Steve Carroll, Patrick Dicker, Elizabeth Tully, Fergal Malone

3. Correlation between histomorphometric placental characteristics and fetal growth restriction in dichorionic twins.
   Etaoin Kent, Angel Mthunzi, Aiveen O’Malley, John Gillan, Fionnuala Breathnach, Fionnuala McAuliffe, Michael Geary, Sean Daly, John Higgins, James Dornan, John Morrison, Gerard Burke, Shane Higgins, Rhona Mahony, Patrick Dicker, Fiona Manning, Fergal Malone

4. Accuracy of sonographic determination of placental cord insertion site in twin pregnancies
Junior Obstetrics and Gynaecology Society Annual Meeting, 2010

Publications

1. Placental pathology, birthweight discordance and growth restriction in twin pregnancy: results of the ESPRiT Study.

2. Placental cord insertion and birthweight discordance in twin pregnancies: results of the national prospective ESPRiT Study.
Placental pathology, birthweight discordance, and growth restriction in twin pregnancy: results of the ESPRIT Study

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OBJECTIVE: We sought to evaluate the association between placental histological abnormalities and birthweight discordance and growth restriction in twin pregnancies.

STUDY DESIGN: We performed a multicenter, prospective study of twin pregnancies. Placentas were examined for evidence of infarction, intra-placental hemorrhage, chorangioma, subchorial fibrin, or abnormal fibrin maturation. Association of placental lesions with chronicity, birthweight discordance, and growth restriction were assessed.

RESULTS: In all, 668 twin pairs were studied, 21.1% monochorionic and 78.9% dichorionic. Histological abnormalities were more frequent in placentas of smaller twins of birthweight discordant pairs (P < 0.02) and in placentas of small for gestational age infants (P < 0.001) when compared to controls. The association of placental abnormalities with both birthweight discordance and small for gestational age was significant for dichorionic twins (P < 0.01; 2001, respectively). No such association was seen in monochorionic twins.

CONCLUSION: In a large, prospective, multicenter study, we observed a strong relationship between abnormalities of placental histology and birthweight discordance and growth restriction in dichorionic, but not monochorionic, twin pregnancies.

Key words: birthweight discordance, placental infarct, placental pathology, intra-placental hemorrhage, subchorial fibrin, twin pregnancy

rates of perinatal morbidity and mortality than singleton pregnancies. While this is, to a large extent, related to the high rate of preterm delivery in these pregnancies, the excess of fetal growth abnormalities is also significant. Independent of gestational age at delivery, twins with significant birthweight discordance have poorer perinatal outcomes. The etiology of birthweight discordance in twins has been extensively investigated. In monochorionic twins, differences are largely attributed to twin-to-twin transfusion syndrome, inequalities in distribution of placental mass between the 2 fetuses, and abnormalities in cord insertion site. In dichorionic twins there may be a difference in genetic growth potential in certain cases, but frequently growth discordance is a pathological entity leading to adverse neonatal outcomes.

Placental pathological examination at both a gross and microscopic level is useful in informing our knowledge of the etiology of abnormal growth in both singletons and twin pregnancies, with a variety of placental pathological lesions implicated in intrauterine growth restriction.

In a large prospective cohort of twin pregnancies we evaluated the association of placental pathology with twin growth restriction.

MATERIALS AND METHODS

The Evaluation of Sonographic Predictors of Restricted Growth in Twins (ESPRIT) Study was a prospective, multicenter, observational study of twin pregnancies carried out by the Perinatal Ireland Research Consortium at 8 tertiary-level obstetric units in Ireland from May 2007 through October 2009. The ESPRIT Study was set up with the primary aims of establishing a level of birthweight discordance in twin pregnancies that would serve as an independent predictor of adverse perinatal outcome. The study had a number of prespecified secondary analyses including the evaluation of the role of placental pathology in the etiology of birthweight discordance and restricted growth in twin pregnancies, institutional review board approval was obtained in each center and participants gave written informed consent, including consent to placental histological examination after delivery. Inclusion criteria for the study were twin pregnancies enrolled <22 weeks' gestation, with both twins alive at the time of enrollment and intact membranes. Monochorionic twins were ex-
nt available to carry out the placental examination locally in accordance with the study protocol, the placental examination was carried out in the pathology department of the Rotunda Hospital, Dublin, the coordinating hospital for the study.

Formalin fixation was carried out as per local practices in the delivery hospital. Placentas undergoing fixation were immersed in 10% phosphate-buffered formalin for a minimum of 24 hours.

Gross placental examination was performed to evaluate umbilical cord vessel number and umbilical cord insertion site. Evaluation of placental choriocytosis was performed by examination of the intervillosous membrane. Both gross and histological examinations of the intervillosous membrane were performed and each twin pregnancy was recorded as dichorionic or monochorionic.

The membranes were sampled at the rupture site and at a further random site and examined histologically. A cross-section of each umbilical cord from the placental end and the fetal end was submitted for microscopy. Each placental disc was examined macroscopically and multiple sections from each disc were submitted for histological examination. When examining the monochorionic placentas the vascular equator was identified and the placental mass on either side of this was considered separately and the findings were assigned to the corresponding twin. The following placental pathological lesions were recorded: placental infarction, chorangiosis, subchorial fibrin deposition, and retroplacental hematoma. All lesions identified on gross examination were sampled for histological examination. The size of the lesion was recorded and its location relative to twin 1 or twin 2. The presence of histological abnormalities in placental villus maturation was noted for either twin in each twin pair. The composite outcome of placental abnormalities used in the final analysis was the presence of ≥1 of the placental lesions described.

Birthweight discordance was calculated for each twin pair by expressing the absolute difference in birthweight as a percentage of the birthweight of the larger twin. For the purpose of this analysis a discordance level of ≥20% was deemed significant. Twins were classified as appropriate for gestational age (AGA) or small for gestational age (SGA) by plotting birthweights on twin-specific birthweight centiles. SGA was defined as birthweight <3rd centile for gestational age.

The frequency of placental pathological lesions was compared for monochorionic and dichorionic twin pregnancies. The composite placental pathology was then assessed as a factor in birthweight discordance and growth restriction. Frequency of occurrence was compared between smaller twins of birthweight discordant pairs and the larger co-twin and discordant controls. The relative frequency was also analyzed between twins with birthweights <3rd centile for gestation and those appropriately grown. Analyses were stratified by chorionicity.

Statistical analyses were performed using SAS software (version 9.1; SAS Institute, Cary, NC). Relative frequencies were corrected using the χ² test. Paired stu-
RESULTS
Of 1001 twin pairs recruited to the ESPRIT Study, 66.7% (n = 668) had complete placental pathological examination data available for analyses. Of these, 21.1% (n = 141) were monochorionic and 78.9% (n = 527) dichorionic. Table 1 illustrates the clinical characteristics of the cohort. Monochorionic twins were delivered at an earlier mean gestational age and were on average 302 g lighter than their dichorionic counterparts. The relative frequency of both birthweight discordance of ≥20% and birthweight <5th centile was not statistically significantly different between the 3 groups. A composite measure of adverse perinatal outcome, which included any of the morbidity measures described above or perinatal death, was more frequent in monochorionic twins.

Overall 34.7% (n = 464/1356) of twins in the study group had a placenta that demonstrated ≥1 of the placental pathological lesions assessed. Lesions were more frequently seen in placentas of monochorionic than dichorionic twins (P = .009) (Table 2). When the individual histological abnormalities were categorized all abnormalities other than chorangioma were significantly more common in monochorionic placentas (Table 2).

The relationship between birthweight discordance and placental abnormalities was then analysed. Overall 17.1% (n = 114) of the cohort had ≥20% difference in birthweight between the smaller and larger twin. The results for the smaller discordant twins (n = 114) were compared to the combined group of larger co-twins and twins with concordant birthweight (n = 1222). In all, 44.7% of the smaller twins had abnormal findings at placental examination. This was significantly more frequent than the comparison group of larger twins and twin with concordant birthweight (33.8%, P = .02) (Figure 1).

These results were then stratified by chorionicity to ascertain if this relationship was present in both monochorionic and dichorionic twins (Figure 1). A significant association between abnormalities in the placenta and birthweight discordance was found in the dichorionic cohort, with a higher frequency of placental pathological lesions in the smaller twins, but a similar association was not ascertained among monochorionic twins.

A further analysis of the results was performed to determine the correlation between fetal growth restriction and placental pathology, comparing twins with birthweight <5th centile for gestational age (SGA) and those with birthweight >5th centile (AGA). Of the 6.7% (n = 90) of SGA babies within the cohort, 57.8% (n = 52) had evidence of ≥1 of the described abnormalities of placentaion. This was almost twice as frequent as for AGA (n = 412/1246, 33.3%, P = .001).

When further analyzed separately according to chorionicity the association between SGA status and placental pathology was significant for dichorionic, but not monochorionic, twins (P = .001 and .23, respectively) (Table 2), although the latter analysis was limited by the relatively low number of SGA monochorionic twins (n = 16) in our study cohort.

COMMENT
It is now well established that twin pregnancies with evidence of growth discordance are at increased risk for perinatal mortality and morbidity.¹ Placental histological examination is vital in the evaluation of intrauterine growth restriction. Studies in singletons have shown morphological changes in villus structure in placentas of fetuses affected by intrauterine growth restriction.²⁻⁵ The abnormalities in villus development are a consequence of defective trophoblast invasion and impaired development of the interplacental circulation.
chronic twin pregnancies complicated by growth abnormalities. In our prior study we established a significant association between noncentral placental cord insertion sites and growth abnormalities in monochorionic twins. A similar association was not seen for dichorionic twins. In direct contrast the results presented here establish that for dichorionic twin pregnancies discordance may be related to underlying uteroplacental insufficiency selectively affecting one twin. While there was a high frequency of placental abnormalities observed in monochorionic twins this did not appear to influence the occurrence of birthweight discordance. This finding suggests that any placental lesions in monochorionic twin pregnancies are evenly distributed across the placental disc. The occurrence of significant growth discordance in these pregnancies is likely a factor of an unequal distribution of placental mass between the 2 fetuses, abnormal cord insertion sites, or aberrant vascular connection.

Our study has a number of limitations. The number of placenta unavailable for examination represented one third of the entire study cohort. However, we are satisfied that the 668 twin pregnancies with pathological data available were representative of the overall study group. Complicated twin pregnancies do not appear to be overrepresented in this data set as the proportion of monochorionic and dichorionic twins was equal to that in the overall cohort. Of the total number of twin pregnancies recruited to the ESPRIT Study, 17.2% had >20% birthweight discordance. This is similar to the rate of 17.1% in this subgroup. The composite perinatal morbidity outcome occurred in 17.4% of this group, not significantly different to the rate of 18.2% in the overall cohort (P = .8). There were also no significant differences in gestational age at delivery, mean birthweight, or proportion of SGA twins in the 2 groups.

Another potential limitation is the fact that placental examination was carried out in the delivery hospital rather than in a single pathology laboratory. To minimize differences in placental reporting a detailed protocol for placental examination was designed, the pathological lesions to be assessed were pre-specified and a standardized data collection sheet was used. Other placental pathology findings including signs of inflammation and fetal vascular lesions were inconsistently reported between centers, thus the analysis was limited to the lesions specified in the study protocol. In conclusion, in a large prospectively assessed cohort of monochorionic and dichorionic twin we have established an association between placental pathology and growth abnormalities. Together with our previously published data it adds further depth to our knowledge of the various pathological processes governing growth in twin pregnancies.

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Placental cord insertion and birthweight discordance in twin pregnancies: results of the national prospective ESPRiT Study

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OBJECTIVE: The purpose of this study was to evaluate the impact of noncentral placental cord insertion on birthweight discordance in twins.

STUDY DESIGN: We performed a multicenter, prospective trial of two pregnancies. Placental cord insertion was documented as central, marginal, or velamentous according to a defined protocol. Association of the placental cord insertion site with chorionicity, birthweight discordance, and growth restriction was assessed.

RESULTS: Eighty-one hundred sixteen twin pairs were evaluated. 160 pairs were monochorionic, and 651 pairs were dichorionic. Monochorionic twins had higher rates of marginal (P = .0008) and velamentous (P < .0001) placental cord insertion. Noncentral placental cord insertion was more frequent in smaller twins of discordant pairs than in control pairs (29.8% vs 19.1%; P = .0003). Velamentous placental cord insertion in monochorionic twins was associated significantly with birthweight discordance odds ratio, 3.5; 95% confidence interval, 1.3-9.6; and growth restriction odds ratio, 4.95; confidence interval, 1.1-14.3.

CONCLUSION: Noncentral placental cord insertion contributes to birthweight discordance in monochorionic twin pregnancies. Serologic delineation of placental cord insertion may be of value in antenatal assessment of twin pregnancies.

Key words: birthweight discordance, placental cord insertion, twin pregnancy


Twins are associated with increased perinatal mortality and morbidity rates. Although much of this increase in morbidity is a consequence of the increased rates of preterm delivery in twins, there is also an independent association with growth abnormalities. Studies that have evaluated perinatal outcome in twins have reported excess morbidity and death at increasing levels of birthweight discordance. The higher rates of adverse neonatal outcomes that are associated with discordant growth occur irrespective of gestational age at delivery and independent of small-for-gestational-age (SGA) status. Greater degrees of growth discordance have been shown to increase the risk of intraventricular hemorrhage for both the smaller and larger twin. Placental umbilical cord insertion site has been evaluated as a contributory factor to perinatal morbidity in singletons and twin pregnancies. In singleton pregnancies, a velamentous cord insertion is associated with obstetric complications that include prematurity, congenital anomalies, and fetal growth restriction. A velamentous insertion is found in approximately 1% of singleton pregnancies, with an additional 7% of singleton pregnancies having a marginal cord insertion. Noncentral cord insertion is more common in twins than in singleton pregnancies. In particular, monochorionic twin pregnancies have significantly higher rates of velamentous cord insertions. This has been proposed as a contributory factor in the development of obstetric intrapartum growth restriction in monochorionic twin pregnancies; some studies show conflicting results with respect to the role of velamentous cord insertion in the cause of twins-to-twin transfusion syndrome (TTTS).

The aim of this study was to evaluate the relative frequency of noncentral cord insertion in monochorionic and dichorionic twin pregnancies and to examine the association between noncentral cord insertion and birthweight discordance in twins.

MATERIALS AND METHODS

This study was performed as a prospective secondary analysis of the Evaluation of Sonographic Predictors of Restricted...
Growth in Twins (ESPRIT) study. ESPRIT was a multicenter, prospective, observational study of twin pregnancies that was carried out at 8 tertiary obstetric units in Ireland between May 2007 and October 2009. The primary aim of the ESPRIT study was to establish a threshold for birthweight discordance that serves as an independent predictor of adverse outcome in twin pregnancies. Institutional ethical board approval was obtained in each center, and participants gave written informed consent. Twin pregnancies with 2 viable fetuses that were identified from 11-22 weeks’ gestation were eligible for inclusion. The principal exclusion criteria were monoamniotic and structural or chromosomal abnormalities in either twin. Cases were excluded subsequently if an intrauterine death of 1 or both fetuses occurred at <24 weeks’ gestation.

Study subjects underwent serial sono- graphic assessment of biometric parameters and multivessel Doppler ultrasound studies. Outcome data that were collected included maternal and obstetric characteristics, delivery and birthweight outcomes, and perinatal morbidity and mortality data.

Placental examination was carried out in the pathology department of the delivery hospital, according to a defined protocol. Formalin fixation of the placentas was carried out as per local practice in the delivery hospital. All placentas had chorionicity confirmed with gross and histologic examination of the intertwin membrane. Placental cord insertion site for each twin was recorded as central, marginal, or velamentous. Marginal cord insertion was defined as cord insertion at the edge of the placental disc. Velamentous insertion was defined as umbilical cord insertion into the membranes that are remote from the placental disc. All other cord insertions were defined as central.

All birthweights were recorded, and twins were recorded as SGA when their birthweight was <5th percentile for gestational age. Birthweight discordance was calculated as the absolute difference in birthweight between the twins and was expressed as a percentage of the weight of the larger twin. For the purposes of this analysis, significant birthweight discordance was defined as a difference in birthweight of >20%.

The overall distribution of cord insertion site was compared between monochorionic and dichorionic twin pregnancies. The rate of each type of cord insertion was compared between twins that were SGA and those whose birthweight was appropriate for gestational age. To evaluate the association with birthweight, discordant twins were divided into 3 groups: the lighter twin of birthweight-discordant pairs, the heavier twin of birthweight-discordant pairs, and twins with birthweight discordance <20% (concordant). Prospective risk of SGA status and birthweight discordance with marginal and velamentous cord insertion was calculated.

Statistical analyses were performed with SAS software (version 9.1; SAS Institute Inc, Cary, NC). Relative frequencies were compared with the use of the chi-squared test. The paired Student t test was used to analyze continuous variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate risks and the uncertainty around the risk estimates. A probability value of <.05 was considered statistically significant.

Results

Our thousand twenty-eight twin pregnancies were recruited at 8 tertiary level centers in the ESPRIT study. 1061 patients completed the study and delivered at 1 of the 8 participating study centers. In 165 cases, the placentas were lost to follow-up. In a further 20 cases, placental examination was completed, but the umbilical cord insertion site was not possible to determine or failed to be recorded. This left 936 twin pairs with data available for analysis. Table 1 outlines the clinical characteristics of this cohort: 20.2% of the pairs (n = 188) were monochorionic diamniotic, and 79.8% of the pairs (n = 851) were dichorionic. Monochorionic twins were delivered at a mean gestational age of 34.7 weeks, compared with 36.3 weeks’ gestation for dichorionic twins. Mean birthweight was significantly lighter in the monochorionic cohort when compared with the dichorionic cohort (P = .0001); however, the proportion of SGA infants was similar in both groups (P = .5). Birthweight discordance of >20% was present in 17.3% of twin pregnancies. There was no significant difference in the frequency of birthweight discordance between monochorionic and dichorionic twin pregnancies. 7.8% of the monochorionic twin pregnancies (n = 13) in this cohort were affected by TTTS. Overall, 20% of the 1632 individual twins (n = 327) in the study had monochorionic cord insertion demonstrated on placental pathologic examination. Rates of marginal and velamentous cord insertion were 15.3% (n = 250) and 4.7%
(n = 77), respectively. When the results were stratified by chorionicity, monochorionic twin pregnancies had significantly higher rates of noncentral cord insertion when compared with dichorionic twin pregnancies (OR, 2.2; 95% CI, 1.5–2.9). The difference was significant for both marginal and velamentous cord insertion sites (Table 2).

Noncentral cord insertion sites were significantly more common in the smaller twins of birthweight-discordant pairs when compared with both larger co-twins and twins with concordant birthweights (P = .0025; Figure 1). Birthweight discordance was associated significantly with both marginal (P = .05) and velamentous cord insertion (P = .003), when evaluated within the entire cohort.

Within the cohort of 165 monochorionic twin pregnancies, there were 52 cases of significant birthweight discordance (19.4%). Of these, 50% of the smaller twins (n = 16) from birthweight-discordant pairs had a noncentral amniotic cord insertion. Velamentous cord insertion was documented in 9.4% of monochorionic twins and was significantly more frequent (21.9%) in smaller twins of birthweight-discordant pairs than in larger co-twins or concordant control infants (9.3% and 7.9%, respectively; P = .002; Figure 2).

Of the 165 pairs of monochorionic twins in the study cohort, there were 13 cases of TTTS, which was a rate of 7.8%. There was no difference in the frequency of marginal or velamentous cord insertion in monochorionic twins with TTTS when compared with those without TTTS (Table 3). Marginal cord insertions were found in 19.2% (n = 5) and 22.8% (n = 68) of TTTS and non-TTTS cases, respectively. The frequency of velamentous cord insertion was 7.3% for those with TTTS (n = 2), compared with 9.7% for those without (n = 29). The distribution of noncentral cord insertion sites in those twins with TTTS was equal between donor and recipient twins. In dichorionic twin pregnancies, there was a trend toward increased rates of noncentral placental cord insertion among smaller twins (P = .051).

The frequency of marginal cord insertion in smaller twins was 18.3%, compared with 13.6% in larger co-twins, and 12.9% in the cohort with concordant birthweight (P = .38). Velamentous cord insertion was observed in 5.2% of smaller birthweight-discordant twins, 2.8% of larger birthweight-discordant twins, and 3.4% of concordant twins (P = .18).

The association between noncentral cord insertion and intrauterine growth restriction was evaluated by comparing frequencies of central, marginal, and velamentous cord insertion in those twins who were SGA and those who were appropriate-for-gestational age (Table 4). Overall, 17.9% of SGA monochorionic twins (n = 11) had a noncentral cord insertion site. Statistically higher frequency of both marginal and velamentous cord insertions were found in SGA monochorionic twins, when compared with appropriate-for-gestational age matched controls (n = 166) (P = .002).
appropriately grown infants. Within the dichorionic cohort, no association was found between cord insertion site and SGA status.

Evaluation of the risk of birthweight discordance for a fetus with noncentral cord insertion gave an overall OR of 1.8 (95% CI, 1.2–2.6). This risk increased with monochorionicity (OR, 2.6; 95% CI, 1.1–5.6) and with the combination of monoamnionicity and a velamentous cord insertion (OR, 3.5; 95% CI, 1.3–9.4; Table 3). The OR for birthweight <3rd percentile for gestational age in monochorionic twins with a noncentral cord insertion is 3.22 (95% CI, 1.26–8.27). A velamentous cord insertion further increases this OR to 4.03 (95% CI, 1.34–14.33).

**COMMENT**

Twin pregnancies pose a unique challenge to obstetric management and are associated with markedly increased rates of perinatal morbidity. This is particularly true of monochorionic twin pregnancies. Growth discordance is associated independently with adverse outcomes, and identification of factors that contribute to growth abnormalities in these pregnancies may improve antenatal risk stratification, with the institution of intense fetal surveillance in high-risk cases.

There are multiple putative mechanisms for discordant growth in twin pregnancies, which differ according to chorionicity. Studies that have evaluated placental factors have suggested that unequal placental sharing and abnormalities of umbilical cord insertion contribute to growth discordance in monochorionic twin pregnancies. An association with noncentral umbilical cord insertion and birthweight discordance in dichorionic twins has been reported less consistently. Most published studies have been retrospective with these prospective studies that selectively have evaluated risk in monochorionic twins only.

In a retrospective analysis of 447 twin pairs, Hanley et al. found that velamentous cord insertion did not predict birthweight discordance in dichorionic twins but was associated with a 13-fold increase in risk of discordance among monochorionic twins. Conversely, Victoria et al. retrospectively evaluated 382 twin pregnancies with respect to growth discordance and placental disease. Umbilical cord abnormalities, which were defined as velamentous cord insertion and single umbilical artery, were compared between concordant, mildly discordant, and severely discordant twin pairs. In both dichorionic and monochorionic pregnancies, umbilical cord abnormalities were significantly more frequent in the smaller twins of severely discordant twin pairs than in mildly discordant or concordant twin pairs.

These retrospective studies that evaluated the impact of cord insertion site are supported by 2 prospective studies in monochorionic twins. De Paep et al. evaluated placental characteristics in 216 monochorionic twins without TTTS. Higher rates of velamentous cord insertion were documented among the 36 discordant pairs in this study. The 22% rate of velamentous cord insertion that was found in birthweight discordant monochorionic twins in this study was comparable with the rate of velamentous cord insertion in smaller twins of birthweight discordant pairs in our study. This study also reported significantly increased frequencies of unequal placental sharing in birthweight discordant twins. This provides a putative explanation for the mechanism of birthweight discordance in the presence of an abnormal placental cord insertion.

Machin et al. evaluated 60 monochorionic placentas and found marginal or velamentous cord insertion to be associated with an increased risk of significant birthweight discordance. The rate of velamentous cord insertion in the cohort studied by Machin was extremely high at 43%. The fact that this is significantly higher than the rates that are found in our study and in other published studies indicates that the study sample may not have
been representative of the general monochorionic twin population. Although the results of the study by Machin support the findings of our study, our study represents an unselected cohort of twins; therefore, our results are applicable to any population of twin pregnancies.

We performed a large, multicenter, prospective study of 861 twin pregnancies (335 monochorionic and 526 dichorionic) and found abnormal placental umbilical cord insertion to be associated significantly with discordant growth. We documented high rates of both marginal and velamentous cord insertions in twin pregnancies. Compared with reported frequencies of noncentral cord insertion in singleton pregnancies,12 marginal cord insertion was noted approximately twice as often (13.3% vs approximately 7%) in the twin pregnancies; in our study with velamentous cord insertion, it was approximately 5 times more frequent (4.7% vs approximately 1%). The rates of velamentous cord insertion in our study are somewhat lower than the rates that were reported in a large cohort of twin placentas that were evaluated by Sato and Benirschke.13 In their cohort of 589 monochorionic twins, the rate of velamentous cord insertion was 12% (7.3%) of the 780 dichorionic twins in their study had a velamentous cord insertion. The differences in rates in the 2 studies may be attributable to the fact that, because theirs was a retrospective study, Sato and Benirschke may have had a higher proportion of complicated twin pregnancies, because these placentas are more likely to be retained for pathologic examination.

Overall in our study cohort, the risk of birthweight discordance of +20% was 80% higher for twins with a noncentral cord insertion site. This increase was observed both in twins with a marginal cord insertion and twins with a velamentous cord insertion site, although the risk of growth discordance was markedly greater with the latter. The excesses of monochorionic umbilical cord insertions that were found in the smaller twins of birthweight-discordant twin pairs in our study was driven by the very high rate of velamentous cord insertions in birthweight-discordant monochorionic twins. However, we did not find a similar relationship between umbilical cord insertion and growth discordance in dichorionic twin pregnancies. Similarly, the association between noncentral cord insertion and growth restriction, as defined by birthweight of <3rd percentile for gestational age, was observed only in monochorionic twin pregnancies.

The results of this study point to a role for velamentous cord insertion in the cause of growth restriction in monochorionic twin pregnancies. Because there was no difference noted in the relative rates of marginal and velamentous cord insertion in TTS and non-TTS monochorionic twin pregnancies, it would appear that this effect is independent of the role of TTS in birthweight discordance. The study by Sato and Benirschke13 evaluated the role of fetal vessel thrombi in the cause of growth discordance in monochorionic twin pregnancies. They concluded that thrombi were observed more frequently in placentas with a velamentous cord insertion. The increased risk of fetal vessel thrombi in these cases may be a result of the increased risk of birthweight discordance and birthweight restriction that were observed in monochorionic twins with a velamentous placental cord insertion site. The association between velamentous cord insertion and unequal placental sharing, as studied by De Paep et al.,14 provides further possible explanation for the association that we have found between velamentous cord insertion and birthweight discordance.

Several possible limitations to our study are acknowledged. The number of placentas that did not undergo examination raises the possibility that there is an overrepresentation of complicated twin pregnancies in our sample. However, the proportions of monochorionic and dichorionic twin pregnancies and the rates of both birthweight discordance and

**Table 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Twin-twin transfusion syndrome, n (%)</th>
<th>No twin-twin transfusion syndrome, n (%)</th>
<th>P-value*</th>
</tr>
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<tr>
<td>Twins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>192</td>
<td></td>
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<tr>
<td>Cord insertion site</td>
<td></td>
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<tr>
<td>Central</td>
<td>19 (73.5)</td>
<td>207 (68.5)</td>
<td>.76</td>
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<tr>
<td>Marginal</td>
<td>5 (19.2)</td>
<td>48 (16.4)</td>
<td>.9</td>
</tr>
<tr>
<td>Velamentous</td>
<td>2 (7.7)</td>
<td>29 (9.5)</td>
<td>.75</td>
</tr>
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</table>

*Two-tailed t-test.

**Table 4**

<table>
<thead>
<tr>
<th>Twins</th>
<th>Appropriate for gestational age, n (%)</th>
<th>Small for gestational age, n (%)</th>
<th>P-value*</th>
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</thead>
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<tr>
<td>Monochorionic</td>
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<tr>
<td>Marginal</td>
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<td>Dichorionic</td>
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<tr>
<td>Marginal</td>
<td>162 (13.4)</td>
<td>14 (1.5)</td>
<td>.04</td>
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<tr>
<td>Velamentous</td>
<td>41 (3.4)</td>
<td>5 (0.5)</td>
<td>.26</td>
</tr>
</tbody>
</table>

*Two-tailed t-test.
SGA status are comparable, in the cohort of 816 twins reported here, with the entire cohort of 1001 twin pregnancies that completed the ESFRIT study. The fact that all placental examinations were not carried out in a single laboratory is another potential source of bias. However, the nature of the placental examination variable that was studied here (cord insertion site) should limit any potential bias in this regard.

The utility of sonographic assessment in the determination of the cord insertion site has been examined in singleton and twin pregnancies. Di Salvo et al. compared prenatal sonographic examination and postnatal histopathologic findings with respect to umbilical cord insertion in 38 singleton and 8 twin pregnancies. Forty-nine of the 54 cord insertion sites were delineated correctly with ultrasound scanning, with an overall sensitivity and specificity of antenatal ultrasound finding of 69% and 100%, respectively. The ability of a combination of gray-scale and color Doppler ultrasound scans to identify velamentous cord insertion was evaluated prospectively by Segal et al. in 832 singleton pregnancies. They determined that velamentous cord insertion could be determined reliably with prenatal sonography, with successful visualization of the placental cord insertion site in 99% of cases.

Antenatal surveillance in twin pregnancies aims to identify those pregnancies that are at an increased risk of complications. Although sonographic estimation of fetal weight is known to be a reliable tool in the prediction of birthweight, there is an acknowledged margin of error of up to 20%. Therefore, the addition of other sonographic predictors of birthweight discordance (such as placental umbilical cord insertion site) may be useful in risk stratification and planning of surveillance and management of twin pregnancies.

In summary, this is one of the largest prospective studies to date to evaluate the risk of birthweight discordance that is associated with noncentral cord insertion in monochorionic and dichorionic twin pregnancies. We have found the cord insertion site to be associated with discordant growth and intrauterine growth restriction in monochorionic twin pregnancies. A similar association was not demonstrated in dichorionic twins. Sonographic evaluation of cord insertion site should be considered in the antenatal assessment of twin pregnancies with the finding of a noncentral cord insertion prompting more intensified antenatal fetal surveillance. However, further prospective examination of the accuracy of antenatal ultrasound imaging in this setting is warranted.

REFERENCES