NEURODEVELOPMENTAL OUTCOME FOR TWINS OF THE ESPRiT STUDY:

THE NOTES STUDY

MD THESIS 2021

PRESENTED BY

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DECLARATION

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Full and informed consent for participation in the study was obtained from the parents of all children who were included in the NOTES Study.

_____________________
Cecilie Halling
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CHAPTER 1: INTRODUCTION
TWINS IN HISTORY

Throughout history, the concept of twinning has been viewed as a mysterious, highly fascinating phenomenon. The fact that a woman can bring forth not one but two children at once is nothing short of miraculous and has been focused on and explored throughout mythology, religion, stories, and plays.

Famous twin gods exist in Greek and Roman mythology. These twins are often portrayed as strong, compassionate, and enlightened. In Greek mythology, they are the sons of Zeus and are known as the Dioscuri; their names are Castor and Pollux, and they are present in both Greek and Roman mythology where it is claimed they fought for the Romans against the Latins (Falkner 1968). The influence of these twin gods is still witnessed today as made evident by the number of sculptures and pictures depicting them (Corsello 2013).

Twins were not always idolised in the world of mythology. Another common theme is the abandonment of twin gods. Perhaps the most famous example is that of the Roman twins Romulus and Remus. Sons of Mars, the God of War, and Rhea Silvia, a vestal virgin, the twins were put in a basket and thrown into the Tiber River shortly after birth. They did not drown as was hoped but were instead rescued by a she-dog who fed the babies with her own milk and cared for them. Legend states that the city of Rome was probably founded by Remus and Romulus. After killing his brother Remus, Romulus became the first
king of Rome in 753 BC (Falkner 1968). Viewing twins negatively has also been witnessed in different civilizations throughout time. The Yoruba communities (mostly present in Nigeria) for example, viewed twins as a “curse” or a “divine punishment”, comparing them to animals, for whom twin births are very common (Corsello 2013).

Throughout history, twins have been held in high regard in different religions. Vedic, the oldest known Indian religion, had 2 divinities, 2 young twin gods known as Asvin. The twins represented all that was good and were known to work miracles. Christian religions as well make reference to twins. The Bible tells the story of Rebekah, who was barren. God took pity on her and blessed her with twins (Falkner 1968).

Mythology and religion are not alone in paying tribute to twins. Playwrights such as William Shakespeare devoted two plays to the twin theme. “The Comedy of Errors” tells the story of identical twins separated at birth, and “The Twelfth Night” tells the story of non-identical twins, Viola and Sebastian. Shakespeare more than likely drew his inspiration for his twin themes from his own twin daughters, Hamnet and Judith (Falkner 1968).

TWINS IN RESEARCH

In 1883, Sir Francis Galton, cousin of Charles Darwin, published the book “Inquires Into Human Faculty and its Development,” a lengthy account into the psychology of human intelligence. Through this work,
Sir Galton introduced the idea of nature versus nurture and applied it to human development. For this purpose, he recognised the potential twins, in particular identical twins, have as a valuable research tool. Put simply, “If identical twins are different for any factor, this difference must be caused by an environmental influence” (Galton 1883). Although overly simplified, this marked the beginning of using twin studies as a “valuable tool for assessing the influence of genetic factors on human trait variation” (Joseph 2004). This was the start of the eugenics movement, “a bio-social movement which advocates the use of practices aimed at improving the genetic composition of a human population” (Galton 1904).

Twin research had sinister beginnings. By the 1920s and 1930s, Germany was the world centre for twin research, using eugenics and “evidence” collected from twin studies on genetics and hereditary traits to scientifically “justify” the killing of mentally ill patients and, later, the Holocaust (Joseph 2004). The “racial hygiene” movement pushed hard for eugenic sterilisation laws, and in 1936 Heinrich Kranz and Friedrich Stumpfl, the authors of two German twin studies of criminality, called for the compulsory castration of all criminals and “undesired” people. In short, as summarised by journalist Lawrence Wright, “It is certainly true that the history of twin research is one of the most appalling chapters in science, having been born in Galton’s aristocratic notions of the natural worthiness of the English upper class, taken to its evil extreme by eugenicists, and too readily used by American scientists to rationalise racial injustice” (Joseph 2004).
INCIDENCE OF TWINNING AND IVF

The incidence of twinning in Ireland has increased from 12.2 per 1,000 live births a year in 1989 to 16.2 per 1,000 live births a year in 2009 (Central Statistics Office, 2010). As of 2016, births in Ireland had dropped by 15.1% since 2010, but the rate of twins had significantly increased with a twinning rate of 19.0 per 1,000 live births (Central Statistics Office 2018). This was down only slightly in 2017 to 18.7 per 1,000 live births (Central Statistics Office 2019). There are several plausible explanations for this overall increase in twin births. Older women who conceive naturally are more likely to conceive twins. Over the past few decades, the age of first-time Irish mothers has steadily increased from 28.0 years in 1990 to 31.7 years in 2010 (Central Statistics Office 2010). This continued to climb, and by 2017 the age of first-time mothers was 32.8 (Central Statistics Office 2019). Another plausible explanation is the increase in the use of assisted reproductive techniques such as IVF (in-vitro fertilisation). From its initial success in 1978 when the first “test tube” baby was born, about 2,500 to 3,000 Irish births a year are as a result of IVF. In 2005, about 18% to 25% of these pregnancies resulted in twins. This is in keeping with European standards as was demonstrated in 2005 through the ESHRE Report (European IVF Monitoring Programme for the European Society of Human Reproduction and Embryology) which reported a 24.7% chance of giving birth to twins following successful IVF (ESHRE report 2005). However, with single-embryo transfers continuing to climb (from 11% in 1997 to above 40% in 2016), there has been a downward trend of
IVF pregnancies resulting in twin births (down to 15% in 2016) (ESHRE report 2016).

IVF also has the potential to increase a woman’s risk of having a small baby or a baby with a major birth defect. In 2002, an Australian study published by The New England Journal of Medicine found a 9% rate of major birth defects in IVF babies compared to 4.2% in babies who were conceived naturally (Hansen 2002). An American study in the same year quoted a 2.3% increased risk of having an LBW (low birth weight) baby in term singletons resulting from IVF (Schieve 2002).

THE PLACENTA AND ITS DEVELOPMENT

The word placenta means “a cake” and refers to the discoid shape of the placenta (Fitzgerald 1994). It begins to develop upon implantation of the blastocyst into the maternal endometrium. This occurs at about six to seven days after conception (Polin 2011).

The blastocyst is the name given to the embryo five days post conception and consists of an outer layer called the trophoblast and an inner layer called the embryoblast (Polin 2011). The trophoblast will form the placenta and the embryoblast will form the entire embryo (Fitzgerald 1994). Loosely arranged, branching cells called the hypoblast make up the cavity of the blastocyst and these cells constitute the extraembryonic mesoderm. The chorionic mesoderm is the internal surface of the trophoblast lined with hypoblasts (Fitzgerald 1994).
As early as twelve days post conception, the trophoblast erodes the maternal endometrial vessels, and a primitive maternal placental circulation is formed (Polin 2011). At this time, the trophoblastic epithelium becomes the cytotrophoblast and the overlying layer becomes the syncytiotrophoblast. The syncytiotrophoblast secretes enzymes that help sustain the pregnancy (Fitzgerald 1994). Stems of the cytotrophoblast divide and link up with one another, forming a shell around the blastocyst. Initially these are known as primary villi. Once invaded by chorionic mesoderm, they are known as secondary villi. They become tertiary villi when blood vessels develop within the chorionic mesoderm. Maternal and embryonic blood circulations are separated by villous capillary endothelium and trophoblast (Fitzgerald, 1994). Figure 1 (page 20) illustrates the development of a fertilised ovum until day twenty-three post conception (Zephyris 2010).
A properly functioning placenta is essential for the healthy development of the fetus. A malfunctioning placenta may result in fetal loss, complications such as IUGR (intrauterine growth retardation), and premature delivery leading to increased neonatal mortality and morbidity (Polin 2011) and possibly subsequent long-term neurodevelopmental impairment.

Figure 1: The initial stages of human embryogenesis
CHORION AND AMNION

The chorion and amnion are the two placental membranes. The chorion is formed by the extraembryonic mesoderm and the two layers of the trophoblast. The chorion surrounds the embryo and other membranes (Polin 2011). The chorionic villi arise from the chorion and invade the endometrium, thus allowing maternal-fetal transfer of nutrients (including oxygen) from mother to fetus and excretion of waste products from fetus to mother (Fitzgerald 1994). The amnion is the membrane closest to the fetus and it is in direct contact with the amniotic cavity. The amnion, or fetal surface of the placenta, is smooth and shiny; it is adherent around the placental margins and invests the umbilical cord (Fitzgerald 1994). This is illustrated in figure 2 (embryology.med.unsw.edu.au).

Figure 2: Placental development
CHORIONICITY

Put simply, chorionicity refers to the presence of one or two chorions (and therefore one or two placentas) in a twin pregnancy. One chorion (monochorionic twin pregnancies) results in the trophoblastic shell giving rise to a single placenta. Two chorions (dichorionic twin pregnancies) result in the production of two separate embryoblasts and trophoblasts, resulting in each fetus having its own placenta, chorionic sac, and amniotic sac (Fitzgerald 1994).

Over 70% of twins are dizygotic or non-identical (Fitzgerald 1994). Dizygotic twins are the result of fertilisation of two simultaneously released ova by two spermatozoa. These twins are like any other siblings and may be either different genders or the same gender.

Monozygotic, or identical twins, result from fertilisation of a single ovum. Thus, these twins result from a single zygote and will have the same genotype. They will therefore always be the same gender. A single zygote may give rise to twins in three different ways (Fitzgerald 1994):

1. Dichorionic, diamniotic twins (DCDA): Although usually resulting in dizygotic twins, about 10% of DC twins are monozygotic or identical; the remainder will be dizygotic or non-identical. DCDA monozygotic twins are the result of division of the fertilised ovum before day three post conception (Fitzgerald 1994, Impey 1999).
2. Monochorionic, diamniotic twins (MCDA): The majority of monozygotic twins are MCDA twins (over 70%). In this group, there is duplication of the embryoblast within a single blastocyst (Fitzgerald, 1994). This duplication occurs from day four post conception and results in 1 chorion and placenta and two amniotic sacs.

3. Monochorionic, monoamniotic twins (MCMA): About 4% of monozygotic twins are the result of delayed division (after day seven). These twins share one chorion and placenta and one amniotic sac. Conjoint twins are a very rare group of monoamniotic twins (about 1%) whereby incomplete separation occurs after day thirteen post conception (Impey 1999). MCMA twins will not be discussed in this thesis.

Mechanisms of monozygotic twinning are illustrated in figure 3 (Dufendach 2008).
Figure 3: Mechanisms of monozygotic twinning
DIAGNOSIS OF CHORIONICITY AND ZYGOSITY

Clinically, it is important to diagnose chorionicity as monochorionic twin pregnancies are at increased risk of perinatal morbidity and mortality (Shetty 2005). Ultrasonography is an easy and reliable means of accurately diagnosing chorionicity before fifteen weeks’ gestation. From five to eight weeks’ gestation, visualising two gestational sacs confirms dichorionicity. In monochorionic twin pregnancies, there is no thick membrane between the two twins (Fox 2006).

From 8 to 14 weeks, dichorionic twins may have two discrete placentas. However, if two dichorionic placentas fuse early on, it may be more challenging to diagnose chorionicity (Fox 2006, Levy 2003). In this instance, diagnosis is based on the presence of the lambda sign together with the evaluation of the thickness of the inter-twin membrane and the localisation of the placenta (Levy 2003). The lambda sign is the area where the chorion forms a wedge-shape protrusion into the inter-twin space in two fused placentas in a dichorionic twin pregnancy. If present, it is indicative of a dichorionic pregnancy (Fox 2006). In monochorionic twin pregnancies, the lambda sign is absent as the membrane implants flat on the placenta. In addition, the membrane is thin and may be very difficult to find (Fox 2006).

Although chorionicity may be determined in the first and second trimester, zygosity is not always possible to establish. If a monochorionic twin pregnancy is diagnosed, so is monozygosity. If a
dichorionic twin pregnancy is established and the fetuses are different genders, dyzygosity is the only option. However, if the fetuses of a dichorionic twin pregnancy are the same gender, zygosity cannot be accurately established as about 10% of DC twin pregnancies are monozygotic twins (roughly 25% of monozygotic twins are DC) (Fitzgerald 1994, Impey 1999). Examination of the placenta after delivery has proved unreliable in determining zygosity in this group of twins. As monozygotic twins have identical genomes, the only reliable means of establishing zygosity in same-sex DC twins is with DNA (deoxyribonucleic acid) testing such as PCR (polymerase chain reaction) amplified STR (short tandem repeat) analysis with multiple unlinked loci (Yang 2006).

**TWIN GESTATION - PERINATAL COMPLICATIONS**

A twin gestation is considered high risk for both mother and fetuses. Complications in the mother such as hypertension and pre-eclampsia, anemia, placental abruption, and caesarean section delivery are all more commonly encountered in twin gestations (Blickstein 1997).

Compared to singleton pregnancies, infant perinatal mortality and morbidity rates are three to seven times higher in twin gestations (Hack 2008). This is due to the higher rates of complications encountered in twin pregnancies such as utero-placental insufficiency and extreme preterm delivery which may lead to adverse outcomes such as death, IVH (intraventricular haemorrhage), RDS (respiratory distress
syndrome), inter-twin growth discordance, intrauterine growth restriction, and NEC (necrotising enterocolitis) (Hack 2008).

The risk of haemodynamic imbalance due to vascular anastomoses leading to TTTS (twin-to-twin transfusion syndrome) is a complication unique to monochorionic twins and puts this group at higher risk of mortality when compared to dichorionic twins (Hack 2006). A Dutch study from 2006 (Hack) retrospectively looked at a historical cohort of 651 twin pairs born between 1907 and 1938 and found a perinatal morality rate of 27.7% for MC (monochorionic) twins and 15.8% for dichorionic twins (p<0.001). In 2000, Woo reported mortality rates of monochorionic twins as double that of dichorionic twin pregnancies in a cohort of 182 twin pregnancies between 1993 and 1997.

The in-utero death of one twin is another complication encountered in twin pregnancies with a reported incidence of anywhere from 0.5% to 6.8% (Woo 2000), and 50% to 70% of such fetal losses are in monochorionic pregnancies (Woo 2000). This complication can have severe consequences for both the mother and for the surviving twin. For the mother, the retention of a dead fetus puts her at risk of developing DIC (disseminated intravascular coagulation) (Woo 2000). Although the mechanism is not known, it is possibly the result of a gradual reduction in maternal fibrinogen level, in particular in those pregnancies where the time interval between fetal loss and delivery of the surviving twin exceeds five weeks (Woo 2000). Loss of one twin also puts the mother at risk of going into preterm labour. About 90% of
twin gestations (dichorionic and monochorionic) complicated by single intrauterine death will go on to deliver within three weeks, thus potentially putting the surviving twin at risk for complications associated with extreme prematurity (Woo 2000). In monochorionic twin pregnancies, there are significant additional adverse effects on the surviving twin. Due to vascular anastomosis, the surviving twin may exsanguinate into the dead twin when the blood pressure falls at the time of intrauterine death causing severe anaemia or even death of the co-twin. In surviving co-twins, the effects of abrupt hypotension may lead to hypoxic damage and long-term neurological consequences (Woo 2000). Structural abnormalities that have been observed in the surviving twin include microcephaly, porencephaly, haemorrhagic lesions of white matter, post-haemorrhagic hydrocephalus, and bilateral renal cortical necrosis (Woo 2000).

Complications encountered in twin pregnancies not only give rise to short-term morbidities but can also contribute to long-term neurodevelopmental outcomes. In fact, it is estimated that twin pregnancies are associated with a four-fold increase in neurological morbidity when compared to singleton pregnancies (Fortin 2005). This will be addressed in the sections below.
PREMATURITY

Extreme prematurity has long been recognised as a significant risk factor for later neurodevelopmental delays. This is especially relevant today as the threshold of viability continues to be challenged and infants as young as 22+0 weeks’ gestation are resuscitated in some countries. In 2005, a six-year follow-up of over 300 children from The EPICure Study demonstrated that 22% of surviving 24 and 25 weekers were left with a severe disability, including severe learning problems and disabling CP (cerebral palsy) in 12% (Marlow 2005).

Although the risk of developmental delay is greatest in the very premature group of children, infants born between 32 and 36 weeks (late preterm) may also be at increased risk. This is especially significant in regard to twins as a large proportion of twins will be born at this gestation. This is not just due to spontaneous onset of labour or emergency deliveries but also because it used to be acceptable practice to deliver twins electively around 36 weeks. Despite limited long-term neurodevelopmental outcome data on late preterm children, several studies have suggested that late preterm children are at increased risk for neurodevelopmental disabilities. In 2009, Petrini showed that decreasing gestational age was associated with developmental delay, even for those born between 34 and 36 weeks’ gestation. This study also demonstrated that late preterm infants were 3 times more likely to develop cerebral palsy compared with children born at term (Petrini 2009). In 2009, Morse concluded that infants born at 34+0 weeks’
gestation to 36+6 weeks’ gestation face a 36% higher risk of developmental delay and school-related problems up through the first five years of life compared to term infants (Morse 2009). Similar conclusions were also reached in 2008 by Chyi where primary school outcomes of 970 children born between 32+0 weeks and 36+6 weeks’ gestation were compared to 13,671 school outcomes of full-term children. This study found that even those born at 34 to 36 weeks’ gestation had significantly lower reading scores (p<0.05) and math skills (p<0.05) than the full-term group of children (Chyi 2008).

SMALL FOR GESTATIONAL AGE AND IUGR

The vast majority of premature infants will be of low birth weight (less than 2.5 kg). The ELBW (extremely low birth weight) infants (less than 1,000 g) are usually also extremely premature (less than 30 weeks’ gestation). The infants who are premature but are AGA (appropriately grown for their gestation) will therefore have a low birth weight as a direct consequence of their premature birth. In this particular group of infants, the main risk factor for developmental disability is, thus, prematurity.

IUGR (intrauterine growth retardation), however, refers to the fetus that fails to reach its growth potential in-utero. Regardless of the child’s gestation at birth, this has been identified as a risk factor for possible developmental problems. Most IUGR infants are also SGA (small for gestational age) (weight less than 5th to 10th percentile for gestation).
SGA was defined as birthweights less than the 5th percentile in the ESPRiT Study by using singleton Doubilet norms. The Doubilet norms are generated from a cohort of neonates who had accurate gestational age assignment by first-trimester ultrasonography (Breathnach 2011, Doubilet 1997).

A review article by Shah (2011) summarised long-term neurocognitive outcomes of SGA/IUGR infants. The literature suggests that premature SGA infants beyond 5 years of age are at increased risk of impairment in neuromotor, cognitive, behavioural, and scholastic attainments compared with preterm non-SGA infants. Term-SGA infants had problems in scholastic/vocational attainments compared with term non-SGA infants (Shah 2011).

Twins are at a significant risk of IUGR/SGA. This can be due to numerous different intrauterine conditions which, in turn, also puts them at an increased risk for both emergency and elective premature delivery. A small number of studies have attempted to compare growth and development between SGA twins and SGA singletons. There is no evidence to suggest that SGA twins are at higher long-term neurodevelopmental disadvantage when compared to SGA singletons. In 1997, Chaudhari concluded that although SGA twins were lighter and shorter than control singletons at four years, there was no difference in development of SGA twins compared to SGA singletons (Chaudhari 1997).
INTRODUCTION TO GROWTH DISCORDANCE AND DEVELOPMENT

Growth discordance is a recognised complication in twin pregnancies and can occur in both MCDA and DCDA twin pregnancies. In part, it may be due to the inability of the uterine environment to provide for the increased demands of multiple fetuses (Blickstein 1988). Most studies use 20% as the cut-off for “significant” weight discordance, although some studies have used figures as low as 15%.

Studies on singleton infants with IUGR may be confounded by post-natal environmental influences. If growth discordance between twins is due to intrauterine growth restriction of the smaller twin rather than genetic factors, this could negatively impact their development. Using monozygotic twins to minimise the effect of confounding factors, Edmonds et al. (2010) demonstrated that reduced intrauterine growth appears to have a negative impact on cognition throughout childhood (Edmonds 2010). In addition, Boghossian recently (2019) reported on 4,057 very low birth weight twins born in the United States between 1994 and 2011 and found severe growth discordance (over 30%) had a negative impact on cognition in both the smaller and the larger twin (Boghossian 2019).

Most studies investigating neurodevelopmental outcomes of GD (growth discordant) twins, however, are small. As a result, the majority of studies have failed to demonstrate a link between growth discordance
and later neurodevelopmental delays. In 2003, Goyen looked at 21 pairs of growth-discordant VLBW (very low birth weight) premature twins (15% difference in birth weight) and demonstrated that the smaller twin scored significantly lower in the locomotor subscale than his/her larger co-twin (Goyen 2003). A small study by Ross in 2012 examined physical growth and intelligence at three years of age of twenty-six concordant pairs of twins and sixteen pairs of growth-discordant pairs. The smaller twin in the growth-discordant pair performed significantly worse in verbal communication scores (P<0.005) and performance scores (P<0.03) while there was no significant difference found in intra-twin scores between the smaller and larger twin in the concordant group (Ross 2012). More recently, Swamy (2018) reported on sixty-six pairs of growth-discordant monochorionic twins and found that the lighter twin remained at a neurocognitive disadvantage compared to the larger co-twin with mathematics and memory skills showing the largest difference.

A few small studies have looked at long-term neurodevelopmental studies in growth discordant pairs where 1 twin is SGA and the other is appropriately grown. In 2004, Monset-Couchard looked at 36 sets of twins where the smaller twin was SGA and the larger twin was AGA. The smaller twin remained smaller at three years of age and also had more visual (15 vs 11), behavioural (14 vs 5), and speech (14 vs 8) problems but most maintained grade-level parity with their AGA siblings (Monset-Couchard 2004). In 2005, Yinon looked at growth restriction as a determinant of outcome in preterm discordant twins.
This study concluded that premature SGA discordant twin pairs have a 7.7-fold greater risk for neonatal morbidity including IVH, RDS, and NEC when compared to premature AGA discordant twin pairs (Yinon 2005).

**GROWTH DISCORDANCE IN MCDA TWINS**

Twin-to-twin transfusion syndrome is unique to monochorionic twin pregnancies and is seen in about 10% to 15% of these pregnancies (Selman 2011). The condition is responsible for the majority of cases of growth discordance in MCDA twins. There is plenty of evidence to support that monochorionic twins are at increased risk for adverse perinatal morbidity and mortality when compared to dichorionic twins. MCDA twin pregnancies, for example, are associated with up to a ten-fold increase in fetal loss rates, perinatal mortality and morbidity (Selman 2011). Monochorionic twins have higher rates of extreme prematurity and, thus, have higher rates of RDS and NEC (Hack 2008).

Twin-to-twin transfusion syndrome is placental in origin and occurs when the blood flow of the arteriovenous anastomoses in the shared placenta become “unidirectional” (Selman 2011). The degree of twin-to-twin transfusion depends on the number and size of the unidirectional placental vascular anastomoses (Woo 2000). Once established, twin-to-twin transfusion syndrome leads to hypoperfusion with associated oliguria and oligohydramnios of one twin (known as the donor twin) and hyperperfusion with polyuria and polyhydramnios
of the other twin (known as the recipient twin) (Selman 2011). The decreased blood volume of the donor twin leads to anaemia and restricted growth while the recipient twin develops polycythaemia and heart failure secondary to increased blood volume. Eventually, this will also lead to significant growth discordance between the twins. In this situation, the risk of developing cerebral palsy has been found to be as high as 19% compared to the 4% risk of those MC twins with mild chronic TTTS without discordant growth (Adeqbite 2004). In severe TTTS, the treatment of choice is fetoscopic laser ablation of the placental vascular anastomoses. Without treatment, over 90% of pregnancies with severe TTTS will end in fetal demise by 26 weeks’ gestation (Selman 2011). Fetoscopic laser ablation not only increases survival rates of both twins, but studies have also demonstrated a considerable improvement in neurodevelopmental outcome in early childhood. In 2009, Hack reported a reduction in adverse neurodevelopmental outcome from 22% to 26% down to 6% to 17% in TTTS treated with laser coagulation compared to those that were not treated with laser.

Survivors of MCDA twin pregnancies complicated by TTTS are at high risk for later adverse neurodevelopmental outcomes. In an uncomplicated MCDA twin pregnancy where TTTS does not occur, the neurological outcome was thought to be similar to DCDA twins. In 2009, however, Hack was able to demonstrate a significantly higher incidence of mildly delayed development of hearing and language in
uncomplicated MC twins at two years of age compared to paired DC twins.

**DCDA TWINS AND GROWTH DISCORDANCE**

Growth discrepancy is witnessed in monochorionic twin pregnancies and also in dichorionic twin pregnancies. The majority of fetuses in DCDA pregnancies are nonidentical. Because these children are not genetically identical, defining growth discordance in this group is not straightforward. Genetic variance in addition to the in-utero environment may be contributing factors. Certainly, a difference in BW (birth weight) of 10% can be considered normal (Breathnach 2011, Patterson 1990, Blickstein 1989).

Excluding chromosomal abnormalities or congenital infections in only one twin, placental abnormalities are an important cause of growth discordance in dichorionic twins. Placental crowding may occur causing one of the placentas to be disproportionately small compared to the other placenta. This results in restricted growth in one of the twins. Cord abnormalities, such as velaeventous insertion of the cord (umbilical veins traverse the membranes before they come together into the umbilical cord), may also cause discordant growth between the two twins (Hagihara 2003). Dichorionic placentas also sometimes fuse together, which on occasion may lead to vascular anastamosis/communication similar to TTTS seen in monochorionic twins. This situation may lead to growth discrepancy between the two
twins; however, communications large enough to induce marked transplacental flow have not been reported in DCDA twins (Hagihara 2003).

**SINGLE FETAL DEMISE AND DEVELOPMENT**

Single fetal death is common in twin pregnancies. This is particularly true in monochorionic twin pregnancies. In 2011, Mahoney concluded that single fetal death is three times higher in monochorionic twin pregnancies than in dichorionic twin pregnancies (Mahoney 2011). Prior to 30 weeks’ gestation, severe TTTS is a common recognised cause. After 32 weeks’ gestation, Hack found an eight-fold increase in the rate of single fetal deaths in monochorionic twins compared to dichorionic twins (Hack 2008). The same article also stated that 2.9% of the single fetal demise events in the MCDA group were unexplained with no evidence of TTTS or intrauterine growth restriction. Breathnach also found a 1.5% risk of unexplained late (after 34 weeks’ gestation) in-utero death in otherwise uncomplicated MCDA twin pregnancies (Breathnach 2012).

Fetal demise in the first trimester is relatively common and does not appear to be associated with increased perinatal morbidity or long-term neurodevelopmental problems in the surviving twin. Single fetal death in dichorionic twin pregnancies does not have adverse effects on mortality and neurodevelopmental morbidity. When fetal demise of one twin occurs in MCDA pregnancies in the second or third trimester,
however, it has major neurodevelopmental implications for the surviving twin. This is most likely due to the fact that dichorionic fetuses rarely have placental vascular anastomosis, whereas in the monochorionic placenta it is estimated that 85% to 98% have vascular connections (Woo 2000). Following the death of a co-twin in a MCDA pregnancy, it is possible for the surviving twin to exsanguinate into the dead twin through placental connections when the blood pressure drops at the time of fetal death (Woo 2000). If the co-twin survives, the abrupt drop in blood pressure and severe haemodynamic changes may result in ischaemic brain damage and later cyst formation in the brain of the surviving twin (Woo 2000). The risk of later neurodevelopmental impairment in this group has been found to be as high as 60% (Adeqbite 2004). In 2009, Hack found that one in four surviving monochorionic twins developed cerebral palsy, whereas none of the surviving dichorionic twins in this study developed cerebral palsy (Hack 2009).

HISTORICAL REVIEW OF DEVELOPMENTAL SCREENING TOOLS

Developmental surveillance has been defined as a “flexible, continuous process whereby knowledgeable professionals perform skilled observations of children during the provision of health care. The components of developmental surveillance include eliciting and attending to parental concerns, obtaining a relevant developmental history, making accurate and informative observations of children, and sharing opinions and concerns with other relevant professionals”
The goal of developmental surveillance is to enable early identification of children with developmental delays and to intervene early so as to lessen impact on the functioning of the child and family (Committee on children with disabilities 2001).

In the late 1800s, there was a growing concern for the mentally deficient and insane. Child development at this time consisted of single-child case studies (often children of the scientists) and although insufficient for establishing normal child development, these case studies were the basis of the creation of developmental tests in the 20th century (Martin 2007).

Structured developmental surveillance can be traced back to the early 1900s. At this time, the concept of measuring developmental progress in infants and young children was closely linked with attempting to establish criteria for schools for children with intellectual deficiencies (Martin 2007). The concept of mental age and a test to measure it was first published in 1905 by Binet and Simon (Martin 2007). Shortly thereafter, this was revised by Terman who created the intelligence quotient (IQ), which is a ratio between mental age and chronological age multiplied by 100 (Martin 2007).

In the decades (1920s to 1940s) that followed Terman’s development of “IQ,” two basic approaches were used in studying child development. One sought to extend downwards the IQ ladder, to start
with the development of preschool children and to work backwards towards infancy, while the other model began with the newborn infant and extended upwards (Martin 2007). By the 1920s and 1930s, several “IQ” or cognition tests for young children had emerged. One example was the Cattell Infant Intelligence Scale. This used the “downward IQ ladder model,” and although initial studies were encouraging, the ability of such tests to predict an infant’s or young child’s later IQ was weak (Martin 2007). Around the same time, work at Yale University under the direction of Arnold Gesell focused on documenting normal development in young children instead of identifying deviant patterns of development as had previously been the practice (Martin 2007). Gesell also put forth the idea that development occurs simultaneously in many distinct but interrelated domains and not just as a singular factor of intellectual ability (Martin 2007). He also recognised that IQ is not necessarily a static and stable trait, but that it can be influenced by factors such as the child’s environment (Martin 2007). It was through Gesell’s concepts and developmental tasks that more modern researchers based their work. One such example is Nancy Bayley, who eventually developed *The Bayley Scales of Infant and Toddler Development* in the 1960s and 1970s. The work of Nancy Bayley was also largely responsible for discrediting the idea of IQ as being fixed from infancy in the 1940s to 1960s (Martin 2007).

Nancy Bayley first developed *The California First-Year Mental Scale* and *The California Infant Scale of Motor Development* in the 1930s (Martin 2007). The first *Bayley Scales of Infant Development* combined
Bayley’s original first two scales. Unlike prior developmental assessments, *The Bayley Scales* was standardised on larger numbers of infants and norms were based on a nationally representative sample of infants (Martin 2007).

Today it is recognised that the developmental assessment of children in the first few years of life “involves more than the simple administration of a set of developmental test protocols.” It requires the assessor to “blend quiet observation with active probing, to synthesise information from caregivers with that gathered through direct observation of the child, and to be involved in a curious blend of searching for specifically defined responses from a child with inferences based on behavior” (Martin 2007).

In 2009, Kerstjens et al. reported that approximately 5% to 15% of all children in the general population have some degree of developmental delay (Kerstjens 2009); yet in the United States, it is estimated that only about 30% are recognised before school age (Boyle 2011). Due to time, cost, and resource restrictions, it is not feasible to carry out a *Bayley Scales Assessment* (Bayley 2006) on every child. Alternatively, simple and reliable developmental screening tools that can be performed at home by parents are an ideal way to screen all Irish children in the general population for potential developmental delays. An example of such a screening tool is the *Ages and Stages Questionnaires, 3rd Edition (ASQ-3)* (Squires 2009).
From the late 1970s, the United States placed emphasis on the importance of early detection of developmental delays in young children using parents as a primary screening source during the first year of life (Knobloch 1979). As a result, significant research went into developing quick, cost-effective developmental screening tools for use in the low-risk general population. Development of the ASQ first began in the 1980s following evidence that parent-completed developmental questionnaires could reliably be used in a low-risk general population to identify children at risk for developmental delays (Knobloch 1979, Squires 2009). Such questionnaires had lower costs and still maintained a high degree of accuracy at picking up early developmental delays in children when compared to a full developmental and neurologic examination (Gessel) carried out by health care professionals (Knobloch 1979). As a result, the concept of the ASQ-3, initially known as the Infant/Child Monitoring System, was developed in 1980 by Dr Bricker and Dr Squires.

**PERINATAL IRELAND AND THE ESPRIT STUDY**

Perinatal Ireland is an all-Ireland research consortium focusing on carrying out research in the area of women’s and children’s health. The group (the first such consortium in the country) comprises eight of the leading maternal fetal medicine centres and physicians in Ireland and harnesses the expertise of Ireland’s leading maternal fetal medicine community together with a large clinical population. The consortium, funded through a €4.4 million award from the HRB, provides a
specialised research infrastructure including high-end ultrasound imaging equipment and related data and image analysis software, an IT data consolidation network, as well as dedicated research and management personnel (including a biostatistician). Through its eight centres, Perinatal Ireland can access a cohort of over 50,000 patients per year. In addition, supplementary resources such as biological samples, patient data, and associated services can also be accessed. Perinatal Ireland also gives access to a wide range of specialist cross-disciplinary clinical expertise. By translating the findings of targeted basic science research studies, the Perinatal Ireland consortium provides an ideal clinical infrastructure to carry out clinical research studies which may improve the overall quality of clinical obstetric practice.

The ESPRiT Study (Evaluation of Sonographic Predictors of Restricted Growth in Twins) was a multicentre prospective observational study run by Perinatal Ireland across its 8 centres in Ireland. The centres included were:

- The Rotunda Hospital, Dublin
- The Coombe Women and Infants University Hospital, Dublin
- The National Maternity Hospital Dublin, Holles Street
- Our Lady of Lourdes Hospital, Drogheda
- Mid-Western Regional Maternity Hospital, Limerick
- Royal Victoria Maternity Hospital, Belfast
The study aimed to show that inter-twin growth discordance (set at 20%) was attributable to factors other than placental microvascular disease and that factors such as aberrant cord insertion sites and cord vessel number, as identified with antenatal ultrasound, could serve as predictors of birthweight discordance and of adverse perinatal outcome (ESPRiT Study protocol 2007). In addition, the study aimed to establish the level of birthweight discordance at which perinatal morbidity increases in monochorionic and dichorionic twin pregnancy (Breathnach 2011). In 2011, the ESPRiT Study demonstrated that this threshold was 18% in both dichorionic and monochorionic (without TTTS) twins. At this point, there was at least a doubling in risk of adverse perinatal outcome (mortality, HIE, IVH, PVL, NEC, and RDS) (Breathnach 2011).

The ESPRiT Study in Ireland enrolled over 1000 patients over a two-year period (March 2007 to June 2009) with the final patients delivered in October 2009. Eligible patients were those who presented with twin pregnancies from 11+0 weeks’ to 22+0 weeks' gestation with both fetuses alive at the time of enrolment and whose membranes were intact and not in labour. Exclusion criteria included aneuploidy, major structural abnormality in either twin; twin-reversed arterial perfusion sequence or monoamnioncity. Over the two-year period, the cohort of women with twin pregnancies were recruited and placental
morphology/stereology and Doppler indices were compared across 3 groups:

- Group 1: The smaller twin of discordant twin pregnancy (discordance expressed as 20% value of weight of larger twin)
- Group 2: The larger twin of discordant twin pregnancy
- Group 3: The concordant control twins

**DEFINING INTER-TWIN GROWTH DISCORDANCE**

The cut-off for significant growth-discordance between twins is difficult to define. Previous studies have defined significant inter-twin growth discordance anywhere from 15% to 40% (Redman 2002, Cooperstock 2000, Cheung 1995). Redman (2002) found that clinically significant adverse outcomes are not seen until the level of growth discordance reaches 30% to 40%. These studies have shown that this level of discordance is associated with higher rates of NICU admissions, low five-minute Apgar scores, fetal hypoxia, and higher rates of emergency caesarian sections for fetal distress.

The main goal of the NOTES Study (Neurodevelopmental Outcome for Twins of the ESPRiT Study) was to investigate neurodevelopmental outcomes in significant birth-weight discordant twins. The ESPRiT Study initially used a cut-off of 20% to define significant growth discordance (ESPRiT protocol 2007). The NOTES Study therefore
used a birthweight difference of 20% as a minimum cut-off for the growth discordant group of twins.

SUMMARY

In summary, twins have played pivotal roles in multiple areas throughout history including mythology, religion as well as science. Although twin research has a sinister past in Germany in the 1920s and 1930s, there is no denying the unique perspective that twin research provides and this type of research has previously been described as “the perfect natural experiment to separate familial resemblance from genetic influence” when comparing monozygotic twin pairs to dizygotic twin pairs (Martin 1997).

The NOTES Study represents one of the largest studies undertaken to investigate neurodevelopmental outcome of growth-discordant twins. The majority of other twin studies are limited by their small numbers. With over 100 growth-discordant twin pairs participating, this study is uniquely placed in allowing examination of growth discordance as an independent risk factor for adverse developmental outcomes as well as examining other factors that may contribute such as chorionicity, IUGR/SGA, and prematurity. Quantifying the risk of growth discordance on cognitive outcome was also compared to the effects of prematurity. In addition, one of the secondary aims was to investigate the accuracy of the ASQ-3 (Ages and Stages Questionnaire, 3rd Edition) at correctly identifying those children with potential developmental
concerns as identified through the *Bayley Scales* and to determine if it can safely be used as a screening tool in low-risk populations in Ireland to identify those children who may be at risk for neurodevelopmental delays.

**OBJECTIVES AND HYPOTHESIS OF THE NOTES STUDY**

**Primary Objective**
- To compare neurodevelopmental outcome and physical growth from 24 months’ corrected age to 42 months in growth-discordant twins (MCDA and DC) and growth-concordant twins and to compare the relative adverse effects of prematurity and growth discordance on later cognition by means of a standardised parent questionnaire (*The Ages and Stages Questionnaire, 3rd Edition* or *ASQ-3*), a detailed developmental assessment (*Bayley Scales of Infant and Toddler Development, 3rd Edition*) and the *Child Behaviour Checklist* (Achenbach, 2000).

**Secondary Objectives**
- To examine the effect of chorionicity on neurodevelopmental outcome by comparing monochorionic and dichorionic twin pairs.
- To study the effect of growth restriction on neurodevelopmental outcome by comparing SGA versus AGA growth-discordant twin pairs.
To evaluate the *Ages and Stages Questionnaire* as a screening tool for developmental problems in an Irish population by determining its sensitivity and specificity by correlating questionnaire results with *Bayley Scales Assessment* results.

We hypothesised that the NOTES Study would show that significant growth discordance (20%) in twins (both in dichorionic and monochorionic pregnancies) has a negative impact on both physical growth and neurodevelopmental outcomes in the smaller twin. We also hypothesised that prematurity may have an equally or even greater negative impact on development.
CHAPTER 2: METHODS
The NOTES Study was a cross-sectional multicentre prospective follow-up study of the ESPRiT Study. Upon enrolment to The ESPRiT Study, each patient was consented for further antenatal and post-natal assessment. Parents consented to being contacted again to participate in a further study looking at the long-term neurodevelopmental outcome of their twins.

Ethics approval was obtained at each individual institution. Neonatologists or developmental paediatricians were identified at each hospital as collaborators in the NOTES Study.

**STUDY POPULATION**

All twins (monochorionic and dichorionic, growth discordant and growth concordant) who participated in the ESPRiT Study from the eight academic centres across Ireland were eligible to participate in the NOTES Study.

**EXCLUSION CRITERIA**

Monochorionic-monoamniotic twins (MCMA) and twins with severe twin-to-twin transfusion syndrome (TTTS) requiring laser ablation (prior to 24 weeks) were excluded from the ESPRiT Study at an early stage and were therefore also excluded from the NOTES Study. Twins with TTTS not requiring laser ablation were included in both the ESPRiT Study and the NOTES Study. Additional exclusion criteria for
the NOTES Study were a known diagnosis of autism or postnatally diagnosed chromosomal abnormalities in one or both twins. These children were excluded because both conditions are associated with adverse neurodevelopmental outcomes. In these cases, a poor performance on the Bayley Scales Assessment could be attributed to the underlying diagnosis and it would therefore be difficult to interpret the contribution of other factors, such as growth discordance, to the developmental outcome of the child. In addition, it would be very difficult to obtain reliable scores on the Bayley Scales Assessment in a child with autism as these children would be unable and/or unwilling to follow instructions for the tasks involved in the assessment. Only 3 pairs of twins were excluded from the NOTES Study due to a known diagnosis of autism.

STUDY INSTRUMENTS

ASQ-3

The Ages and Stages Questionnaire, 3rd Edition, or ASQ-3 is a developmental questionnaire that both parents and health care workers can fill out to establish a child’s developmental status. It consists of 21 questionnaires for different age groups between 1 month and 5.5 years of age and contains 30 items covering 5 developmental domains (Squires 2009):

- Communication
- Gross Motor
The ASQ-3 is an easy, reliable screening tool to identify those children in need of a more detailed developmental assessment. Language is simple and illustrations are frequently used to demonstrate a task. This enables parents with limited reading skills to carry out the assessment (Squires 2009).

For each item, there are 3 possible scores. A score of 0 is given if the child is “not yet” able to perform the task, a score of 5 is given if the child “sometimes” performs the task, and a score of 10 is given if the child is able to perform the task. The total scores for each domain are calculated with a maximum score of 60 in each category. Cut-off scores vary depending on the domain and the age of the child. If a child scores lower than 2 SD below the mean in any of the 5 domains, a more detailed developmental assessment is recommended. The results of the ASQ-3 also highlights those scores that fall between 1 to 2 SD below the mean, and it is recommended that repeat ASQ-3 assessments be carried out on these children at regular intervals. The 24 month ASQ-3 questionnaire can be found in the appendix (page 162).
DEVELOPMENT AND VALIDITY OF THE ASQ-3

The validity of a screening tool is considered to be the degree to which the tool measures what it claims to measure. Internal validity refers to the degree to which the investigator draws the correct conclusions from the tool while external validity refers to the degree to which these conclusions can be appropriately applied to other children who were not tested (Hulley 2013). The sensitivity of a screening tool measures the proportion of positives (for example, developmental delay) that are correctly identified as such (true positive rate) while specificity measures the proportion of negatives (for example, children without developmental delay) that are correctly identified as such (true negative rate) (Hulley 2013).

Development of the ASQ-3 began in 1980 by Dr Bricker and Dr Squires. Since then, the questionnaires have been examined closely. To determine the validity, the results obtained on the ASQ-3 have been compared with performances on other standardised developmental tests. In addition, a child’s score based on parent-completed questionnaires were compared with results obtained when the ASQ-3 was carried out by assessment teams (Squires 2009). Scores were found to be consistent between the 2 groups.

Data has been collected on the ASQ-3 since 2002 and over 18,000 questionnaires have been analysed. These questionnaires have been used to establish screening cut-off points (Squires 2009). Overall
external validity has been found to be 86% with a range of 73% to 100% (Squires 2009). The failure rate of the ASQ-3 to identify children with actual developmental delays ranged from 1% to 13%. Identifying children without actual delays was more common and ranged from 6% to 13%. Sensitivity of the ASQ-3 ranged from 85% to 92% and the specificity ranged from 78% to 92%. Although the higher sensitivity means that the ASQ-3 will identify a proportion of children without actual delays, it also minimises the number of “missed” children with potential neurodevelopmental impairments.

Since it was first published in 1995, the Ages and Stages Questionnaires have also been used in many different countries across Europe, South America, and Asia. As a result, studies looking at the validity of the ASQ in countries outside the United States have emerged. In 2013, for example, Armijo published a study looking at the validity of the Chilean version of the ASQ. Parents of 1,572 low-risk term children completed the questionnaire as well as parents of 224 preterm infants (24 to 31 weeks) and 102 parents of children with neurological or genetic diseases. Armijo found that the mean scores in the Chilean version of the ASQ were comparable to United States normative data and concluded that the ASQ is a valid, reliable, and feasible tool for assessing development in children eight to eighteen months in a Chilean urban population (Armijo 2015). In the U.K., the ASQ-3 has been incorporated into the Healthy Child Programme as a developmental screening tool for two- to two and a half-year-olds (Kendall 2014).
The Bayley Scales of Infant and Toddler Development, 3rd Edition, was launched in 2006 and was revised from the 1993 Bayley Scales of Infant Development, 2nd Edition. The Bayley Scales is “an individually administered instrument that assesses the developmental functioning of infants and young children between one month and 42 months, 15 days of age” (Bayley 2006). Due to the complexity of administration and interpretation of results, assessing a child with the Bayley Scales requires a professional such as a research nurse, psychologist, or doctor who has had additional structured training in carrying out and scoring the assessment. The main purpose of the assessment is to identify children with developmental delays (Bayley 2006). The entire assessment booklet can be accessed from pearsonassessments.com

The Bayley Scales, 3rd Edition, assesses infant and toddler development across five domains:

- Cognitive
- Language: Receptive and Expressive
- Motor: Fine and Gross
- Social-Emotional
- Adaptive Behaviour
The cognitive scale examines a child’s cognitive processing including memory, concept formation, exploration, and manipulation. The majority of the cognitive items in the mental scale from the *Bayley Scales, 2nd Edition*, were retained in the 3rd Edition; however, directions to each task were re-written in order to be less reliant on the child’s receptive language skills. Also, those items pertaining more to language and fine motor skills were moved to their respective scales in the *Bayley Scales, 3rd Edition* (Bayley 2006).

The language scale is composed of 2 subsets: receptive communication and expressive communication. Items from the mental scale pertaining primarily to language in the *Bayley Scales 2nd Edition* were moved to the appropriate scale in the *Bayley Scales, 3rd Edition*. In the 3rd Edition, additional items were also adapted from the *Preschool Language Scale, 4th Edition* (Bayley 2006).

The receptive communication scale assesses an infant’s preverbal behaviours and a child’s verbal comprehension such as the ability to carry out instructions, ability to pick out objects or pictures referenced, and ability to understand plural (-s) and tense markings (-ing and -ed) (Bayley 2006).

The expressive communication assesses preverbal communication such as babbling in infants and vocabulary development in children such as naming objects, pictures, colours, and ability to combine words together to form short sentences (Bayley 2006).
The motor scale also consists of two subsets: fine motor and gross motor. Items from the motor scale of the *Bayley Scales, 2nd Edition*, were moved to the appropriate subscale in the 3rd Edition (Bayley 2006).

The fine motor category assesses an infant’s ability to visually track, reach, and grasp items and a child’s ability to manipulate objects in addition to assessing functional hand skills, perceptual-motor integration and motor planning, and speed. The gross motor subset assesses static positioning, balance, and dynamic movement (Bayley 2006). The cognitive, language, and motor scales are carried out by a trained professional in the setting of a formal assessment. The assessment takes roughly sixty minutes to complete. The social-emotional and adaptive behaviour scales are both questionnaires and are designed for the parent or primary care giver to complete.

The social-emotional scale is adapted from the *Greenspan social-emotional growth chart*, a screening questionnaire for infants and young children (Greenspan 2004). The questionnaire assesses social and emotional milestones in children including communicating needs, using emotions in an interactive purposeful manner, and self-regulation and interest in the world (Greenspan 2004).

The adaptive behaviour scale is composed of the items and skill areas from the parent/primary caregiver form of the *adaptive behaviour assessment system, 2nd Edition* (Bayley 2006, Harrison 2003). This
questionnaire assesses the child’s adaptive skills functioning in areas such as communication, social, self-care, health and safety, leisure, and self-direction (Bayley 2006).

**SCORING OF THE BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT, 3RD EDITION**

The results of *The Bayley Scales* (cognitive, language, motor, social-emotional scales, as well as a general adaptive composite score [GAC] for the adaptive behaviour scale) are reported with a standardised score known as the composite score. These scores have a mean and a standard deviation. Composite scores are scaled to a metric with a mean score of 100, a standard deviation of 15, and a range from 40 to 160. Composite scores of 80 to 89 are considered low average, scores of 70 to 79 are considered borderline low, and scores below 70 are considered extremely low (Bayley 2006).

Scaled scores are available for each subset (cognitive, receptive communication, expressive communication, fine motor, gross motor, social-emotional scales, as well as each individual subscale of the adaptive behaviour scale), and they represent a child’s performance relative to his/her same age peers. Scaled scores are available for all subsets with a range of 1 to 19 with a mean of 10 and a standard deviation of 3 (Bayley 2006).
Diagnosing developmental delay is not clear-cut as there is no universally accepted definition (Bayley 2006). One accepted set of criteria to diagnose delay is as follows: 1.5 standard deviations below the mean in two areas of development (equivalent to a scaled score of between 5 and 6 and a composite score of 78) or a score of 2 standard deviations below the mean in one area of development (scaled score of 4 and composite score of 70) (Bayley 2006).

**VALIDITY OF THE BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT, 3RD EDITION**

Normative data for the *Bayley Scales, 3rd Edition*, was collected between January 2004 and October 2004. Since the *Bayley Scales, 2nd Edition*, in 1993, some items had become outdated and scoring needed to be altered to reflect societal changes (Bayley 2006). To accomplish this goal, experimental editions through five general phases were developed. These phases included the following: conceptual developmental phase, pilot phase, national try-out phase, mini-pilot phase, and the standardisation phase (Bayley 2006).

The standardisation phase included 1,700 children ages 16 days to 43 months and 15 days and was stratified on demographic variables including age, sex, parent education level, and geographic location (Bayley 2006). In order to provide evidence of the assessment’s reliability and validity, concurrent studies were conducted looking at split-half reliability as well as test re-test stability (Bayley 2006).
Evidence of validity was shown by various analyses, including a confirmatory factor-analytic study and mean comparisons with matched special groups and children with normal development. Convergent and discriminant validity was provided by correlation studies between the *Bayley Scales, 3rd Edition*, and several other developmental assessment tools including the *Bayley Scales, 2nd Edition*, the *Wechsler Preschool and Primary Scale of Intelligence, 3rd Edition* (Wechsler 2002), *Preschool Language Scale, 4th Edition* (Zimmerman 2002), *Peabody Developmental Motor Scales, 2nd Edition* (Folio 2000), and the *Adaptive Behaviour Assessment System* (Bayley 2006).

With composite scores of under 80, The *Bayley Scales, 3rd Edition*, was found to have a sensitivity of 89% and a specificity of 99% (Moore 2011).

**BEHAVIOUR: SOCIAL-EMOTIONAL AND ADAPTIVE BEHAVIOUR SCALES**

The *Bayley Scales* includes two measurements that assess social-emotional milestones and adaptive behaviour characteristics. These scales are questionnaires completed by the parent or primary caregiver:

- **Social-Emotional Scale**: The social-emotional scale assesses skills such as ability to play make-believe, ability to express needs with words or motions, and ability to describe feelings (Bayley 2006).
Adaptive Behaviour Scale: The adaptive behaviour scale is arranged into ten categories assessing skills within areas such as self-care (takes shoes off, washes hands with soap, and sits on the potty without being held), functional pre-academics (counts three or more objects, names six or more colours, and states his/her age when asked), and health and safety (refrains from putting toys in mouth, avoids touching or playing with dangerous objects, and follows an adult’s instructions to “stop”). Results are presented as general adaptive composite scores (GAC), which is the sum of the scaled scores of the 10 categories (Bayley 2006).

**BEHAVIOUR: THE CHILD BEHAVIOUR CHECKLIST**

The *Child Behaviour Checklist* is a parent- or teacher-completed questionnaire looking at various behavioural and emotional problems in children. First developed for school-age children in 1983 by Thomas Achenbach, several versions have since been created, including the Child Behaviour Checklist for ages 1.5 to 5 years in 1992. This questionnaire contains 100 statements. For each statement, there are three possible scores, 0 (not true), 1 (somewhat or sometimes true), or 2 (very true or often true) (Achenbach 2000). The questionnaire evaluates:

- **Internalising Behaviour:** These items focus on behaviours where children direct emotions and feelings inward, including somatic complaints (tired, aches, nausea and vomiting, headaches, dizziness, and skin and abdominal complaints), anxious/depressed behaviour, and withdrawn behaviour (Donner 2011).
• Externalising Behaviour: These items focus on behaviours such as aggression and delinquent behaviours such as lying, destruction of his/her own things, and disobedient behaviour (Donner 2011).

• Social, Attention, and Thought Problems: These items include being clingy, preferring to play with younger children, and clumsiness (Donner 2011).

• The Child Behaviour Checklist also assesses other behavioural problems, including nail biting, nose and skin picking, whining, and speech problems (Donner 2011).

A copy of the Child Behaviour Checklist can be found in the Appendix (page 169).

VALIDITY OF THE CHILD BEHAVIOUR CHECKLIST

The Child Behaviour Checklist has been translated into 75 different languages and has been used in over 6,000 professional publications (Donner 2011). It is considered to be both a valid and reliable indicator of children’s behavioural and emotional functioning (Donner 2011). The Child Behaviour Checklist has been particularly helpful in identifying children as young as 18 to 24 months with autistic spectrum disorders. An Italian study from 2013, for example, compared 47 children with diagnosed autistic spectrum disorders to 47 toddlers with normal developmental milestones. In this cohort, the Child Behaviour Checklist was clearly able to differentiate children with autism from
children with normal development by correctly identifying autistic characteristics with a 92% sensitivity and a 97% specificity (Narzisi 2013).

RECRUITMENT
Recruitment ran from July 2010 to June 2012. Minimum age for participation in the NOTES Study was 24 months’ corrected age (age corrected if born under 37 weeks’ gestation) and maximum age was 42 months 15 days, which is the older age limit at which the Bayley Scales can be carried out.

THE NOTES STUDY: PHASE ONE
All twins recruited for the ESPRiT Study were eligible for phase one of the NOTES Study. Parents were first approached via telephone by the lead researcher of the NOTES Study or by the sonographer involved with the mother in the ESPRiT Study. Once verbal consent was obtained, an information leaflet, consent form, and two developmental questionnaires (Ages and Stages Questionnaire, 3rd Edition, or ASQ-3) was posted to each participant. A stamped addressed envelope was included in each packet. Parents were asked to complete the developmental questionnaires for twin one and twin two (each twin was given a unique identification number) and return them together with the signed consent form. Every participant received feedback of the results by either email (if no further assessments taking place) or by telephone.
The main purpose of phase one of the NOTES Study was to investigate the reliability of the ASQ-3 questionnaire at identifying children at risk for developmental delay in our cohort and to determine if it can safely be used as a developmental screening tool in an Irish population.

THE NOTES STUDY: PHASE TWO

Once the consent form and the ASQ-3 were returned to the lead researcher, parents of two distinct groups of twins were contacted again to participate in phase two of the NOTES Study.

These two distinct groups were:

- Growth-discordant twin pairs (≥20% birthweight discordance)
- Control Group: Random selection of twin pairs with concordant growth matched for gestation with the growth discordant group of twins in a 1:1 ratio

For ethical reasons, an additional 2 groups also progressed to phase two of the study. These two groups were as follows:

- High-risk concordant twins: These twins were identified through the ASQ-3. Those growth concordant children scoring 2 standard deviations below the mean in one area of development or those scoring between 1 to 2 standard deviations (SD) below the mean in two or more areas of development were viewed as high risk and were therefore invited to attend for further developmental testing in the form of the Bayley Scales. If only one twin was identified
as high risk, his/her co-twin was also assessed. If a child scored between 1 to 2 SD below the mean in just one area of development, he/she was followed up with a repeat ASQ-3 questionnaire six months later.

- **Singletons:** Surviving twins following single fetal demise, neonatal death, or childhood death were also offered further developmental assessment with the Bayley Scales.

Figure 4 (page 67) summarises twin groups for phase one and phase two of the NOTES Study.

Twins proceeding to phase two of the NOTES Study were contacted again via telephone and invited to attend for the *Bayley Scales of Infant and Toddler Development, 3rd Edition*. Each twin pair was assessed locally, either in the outpatient’s department of a participating hospital or at home. Both twins were assessed on the same day. A total of 4 examiners across Ireland carried out all the *Bayley Scales* Assessments. These examiners were all trained professionals (psychologists or research nurses) or the lead researcher. The examiners had no knowledge regarding which group each twin pair belonged to, and they were also unaware of the twins’ birth history and gestational age at birth. The *Bayley Scales Social-Emotional and Adaptive Behaviour Scales* and *Child Behaviour Checklist* were posted to parents prior to the assessment date so that they could be completed at home and returned on the day of assessment.
Each child had their height, weight, and head circumference recorded on the day of their assessment. For home visits, the examiner brought a scale to obtain the child’s weight and a measuring tape to measure the child’s height and head circumference. The child’s height was measured by getting the child to stand up straight against a wall, heels touching the wall. A piece of paper was then placed on the child’s head and the height was measured by placing the measuring tape from the floor up until the point where the paper touched the wall. If the assessment took place in an outpatient clinic setting, the child’s height was obtained in the same fashion. The child’s weight was obtained using a hospital scale.

Each parent received feedback regarding the results of their children’s assessment in the form of a score report and short letter explaining the results. In the event that a developmental delay was identified, the child was referred to the paediatrician identified as collaborator to the NOTES Study at the hospital where the child was born.
STATISTICAL ANALYSIS

The sensitivity and specificity of the ASQ-3 was evaluated by establishing those twins correctly identified as having possible developmental delays by the ASQ-3 (confirmed by a low score in the Bayley Scales) as well as those twins incorrectly identified as having possible developmental delays (confirmed by a normal score in the Bayley Scales). While sensitivity and specificity are characteristics of the specific test, positive and negative predictive values are influenced by the prevalence of developmental delay in the population that is being tested. For example, if there was a high prevalence of developmental delay in our cohort, it would be more likely that children with abnormal ASQ-3 scores actually have developmental delay, while if there was a
very low prevalence of developmental delay in our cohort, it will be less likely that those with abnormal ASQ-3 results actually have developmental delay (Hulley 2013). The total agreement (accuracy) of the ASQ-3 and Bayley Scales was also established.

Assuming 80% statistical power, a sample size of 120 twin pairs per study group in the growth discordant and control group was determined in order to detect a minimum inter-twin difference of two points on a composite score from the Bayley Scales, assuming a standard deviation of eight points within twin pairs and a nominal 5% level of significance.

Composite and scaled scores from the Bayley Scales Assessment across 5 domains (cognitive, language, motor, social-emotional, and adaptive behaviour) and the Child Behaviour Checklist scores were compared between growth-discordant infants (inter-twin analysis) and also between growth-discordant pairs and control pairs. Height, weight, and head circumference were also compared within growth-discordant twin pairs. Z-scores are a measure of how many standard deviations below or above the population mean a measurement is. We therefore measured the Z-scores for weight and head circumference in the growth-discordant twins as a way to compare these measurements to the “normal” population. We used the RCPCH UK-WHO growth charts to establish “normal” mean values for weight and head circumference.

The effect of chorionicity, prematurity, and birth weight (SGA <5th centile) were also analysed. To account for correlations within twin
pairs, paired t-tests were used for inter-twin comparisons while a linear mixed-effects model (with twin pair as a random effect to account for inter-twin correlations) was used for multiple linear regression analysis of developmental outcomes. Growth-discordant status (either control twin pairs or growth-discordant pairs) was included as a factor in the multiple linear regression while chorionicity and prematurity were included as covariates. For comparisons of maternal and twin pair demographics, unpaired t-tests were used.

We assessed inter-rater reliability of the 4 professionals performing the Bayley Scale Assessments using a random effects model. This looks for the percentage of variation attributable to variability between examiners.

As social class may have an effect on developmental outcome, we recorded this in study participants. Social class was defined as the maximum social class of the household (i.e., maximum social class of mother and father). Social class categories were defined according to the Soc90 classification used in the U.K. and Ireland (Central Statistics Office, 2012). The categories were further combined as either professional/skilled (managerial, professional, or technical) or non-professional/unskilled (manual, semi-skilled, or unskilled). We controlled for social class when analysing differences between the growth-discordant group of twins and the control group of twins. We did not control for any other socio-demographic variables.
All statistical analyses and screening of data were performed using the SAS Software® Version 9.1. Graphical descriptions of the data were performed using the R Software version 2.15.
CHAPTER 3: THE NOTES STUDY:
RESULTS
THE NOTES STUDY

The NOTES Study (Neurodevelopmental Outcome for Twins of the ESPRiT Study) was a cross-sectional, multicentre prospective follow-up study investigating neurodevelopmental outcome in a large cohort of twins (ESPRiT cohort).

The ESPRiT Study originally recruited 1,001 women. Nine hundred and seventy-seven women continued the study with both fetuses alive at 24 weeks’ gestation. Four women subsequently withdrew from the ESPRiT Study prior to its completion. The children of 973 women were therefore eligible for the NOTES Study. This included 948 pairs of twins and 25 singletons resulting from twelve single fetal losses after 24 weeks’ gestation, eleven neonatal deaths, and two childhood deaths prior to three years of age. These singletons were included in the NOTES Study but were not analysed together with the twin pairs. A breakdown of children eligible for the NOTES Study by centre is illustrated in table 1 (page 73). The total number of ESPRiT and NOTES Study participants are illustrated in figure 5 (page 74).
Table 1: Breakdown of children eligible for the NOTES Study

<table>
<thead>
<tr>
<th>Number/Hospital</th>
<th>Twin Pairs (N=948)</th>
<th>Singletons (N= 25)</th>
<th>GD&lt;sup&gt;1&lt;/sup&gt; pairs excluding single survivors (N=154)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Rotunda Hospital</td>
<td>223</td>
<td>4</td>
<td>42</td>
</tr>
<tr>
<td>National Maternity Hospital, Holles Street</td>
<td>242</td>
<td>5</td>
<td>37</td>
</tr>
<tr>
<td>Coombe Women and Infants University Hospital</td>
<td>141</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Our Lady of Lourdes Hospital, Drogheda</td>
<td>26</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>University College Hospital, Galway</td>
<td>94</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Cork University Maternity Hospital</td>
<td>100</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Mid-western Regional Maternity Hospital, Limerick</td>
<td>24</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Royal Victoria Maternity Hospital, Belfast</td>
<td>98</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>1</sup>GD- growth discordant
Figure 5: Flow diagram of total number of twins in ESPriT and NOTES Study

1IUD- Intrauterine Death, 2MCDA- Monochorionic, diamniotic, 3DCDA- Dichorionic, diamniotic
The NOTES Study cohort of twins consisted of both monochorionic-diamniotic (MCDA) twins and dichorionic (DC) twins. As shown above, 948 pairs of twins and 25 singletons were thus eligible for inclusion in the NOTES Study. Perinatal morbidity and neonatal data (mortality, admission to NICU, length of stay in NICU, HIE, IVH, PVL, NEC, RDS, and sepsis) were documented in the ESPRiT database.

**PHASE ONE PARTICIPANTS**

A total of 1,921 children (948 twin pairs and 25 singletons) from the ESPRiT Study were alive at two to three years of age and were therefore eligible for phase one of the NOTES Study which consisted of parents assessing their children with the ASQ-3.

A total of 1,304 children (649 pairs of twins and 6 singletons) consented to participate and completed the ASQ-3. This was a 68% follow-up rate of the entire cohort of 1,921 children eligible for participation in the NOTES Study (948 pairs of twins and 25 singletons).

Phase one participants were divided into two groups for the purpose of analysis of the ASQ-3:

- Growth discordant group (119 twin pairs)
- Concordant group (530 twin pairs)
ANALYSIS OF THE ASQ-3 RESULTS

A summary of the results of the ASQ-3 for the entire twin cohort is displayed in table 2 (page 76). The concordant group scores were significantly worse than the growth-discordant group in the personal-social category; otherwise, no significant differences were found between the two groups. No significant difference was found in the ASQ-3 scores between smaller and larger twin when the growth-discordant twin pairs were analysed (table 3, page 77).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Twin Pairs</th>
<th>Mean</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>GD¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>Concordant</td>
<td>119</td>
<td>53.5</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530</td>
<td>51.6</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>Concordant</td>
<td>119</td>
<td>42.6</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530</td>
<td>42.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Fine Motor</td>
<td>GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>Concordant</td>
<td>119</td>
<td>53.1</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530</td>
<td>52.3</td>
<td>10.6</td>
</tr>
<tr>
<td>Gross Motor</td>
<td>GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>Concordant</td>
<td>119</td>
<td>51.4</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530</td>
<td>51.2</td>
<td>10.1</td>
</tr>
<tr>
<td>Problem Solving</td>
<td>GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>Concordant</td>
<td>119</td>
<td>51.8</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530</td>
<td>49.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Personal Social</td>
<td>GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>Concordant</td>
<td>119</td>
<td>51.8</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530</td>
<td>49.5</td>
<td>9.2</td>
</tr>
</tbody>
</table>

¹ GD- growth discordant
Table 3: ASQ-3 results for growth discordant twins: Larger twin versus smaller twin

<table>
<thead>
<tr>
<th>Category</th>
<th>N=119</th>
<th>Mean</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>53.4</td>
<td>9.2</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Small</td>
<td>53.5</td>
<td>8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine Motor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>43.0</td>
<td>13.9</td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>Small</td>
<td>42.1</td>
<td>14.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross Motor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>53.3</td>
<td>8.6</td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>Small</td>
<td>52.8</td>
<td>9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problem Solving</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>51.8</td>
<td>8.8</td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>Small</td>
<td>51.0</td>
<td>9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal-Social</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>51.8</td>
<td>6.9</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Small</td>
<td>51.9</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SENSITIVITY, SPECIFICITY, AND TOTAL AGREEMENT BETWEEN ASQ-3 AND THE BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT, 3RD EDITION

In order to determine the accuracy of the ASQ-3 at identifying “high risk” children in an Irish population, the sensitivity and specificity of the ASQ-3 was calculated as well as the total agreement between the ASQ-3 and the Bayley Scales.

For this purpose, the control group of the NOTES Study was used. These infants were felt to be the lowest risk group in the population studied.
Table 4 (page 78) illustrates how sensitivity, specificity, positive predictive value, negative predictive value, and the total agreement were calculated. Table 5 (page 79) is a 2 x 2 chi-squared table (compares the proportion of subjects in each of two groups that have a dichotomous outcome) illustrating the actual sensitivity, specificity, positive predictive value, and negative predictive value of the ASQ-3. In addition, the total agreement between the ASQ-3 and the Bayley Scales is also illustrated.

Table 4: A 2 x 2 table demonstrating calculation of sensitivity, specificity, positive, and negative value (PPV and NPV)

<table>
<thead>
<tr>
<th>Test positive</th>
<th>Actual positive</th>
<th>Actual negative</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive: a</td>
<td>False positive: b</td>
<td>Positive predictive value: a/a+b</td>
<td></td>
</tr>
<tr>
<td>False negative: c</td>
<td>True negative: d</td>
<td>Negative predictive value: d/c+d</td>
<td></td>
</tr>
<tr>
<td>Sensitivity: a/a+c</td>
<td>Specificity: d/b+d</td>
<td>Accuracy/total agreement: (a+d/a+b+c+d)</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Sensitivity, specificity, PPV, NPV, and total agreement

<table>
<thead>
<tr>
<th>Developmental delay confirmed by Bayley Scales Assessment</th>
<th>Normal Bayley Scales Assessment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ASQ-3 identified child as high risk (abnormal ASQ-3 result)</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>ASQ-3 identified child as low risk (normal ASQ-3 result)</td>
<td>2</td>
<td>171</td>
</tr>
</tbody>
</table>

Sensitivity: 91%  Specificity: 86%  Accuracy / total agreement: 87%

In our cohort, the ASQ-3 has a sensitivity of 91% and a specificity of 86%. This indicates that 91% of children with developmental delay had an abnormal ASQ-3 result (sensitivity) and 86% of children without developmental delays had normal ASQ-3 results (specificity). The positive predictive value (43%) is the percentage of children who had abnormal ASQ-3 results who truly have developmental delay. The negative predictive value is the percentage of children (99%) with normal ASQ-3 results who do not have developmental delay (Hulley 2013).
PHASE TWO PARTICIPANTS

Identified from the ESPRiT Study were 159 pairs of growth-discordant twins (20% or more discordance). At two to three years of age (IUD after 24 weeks in 1 twin, 3 neonatal deaths, and 1 childhood death), 154 pairs were alive. An additional four pairs were excluded due to confirmed autism (three pairs) or chromosomal abnormalities (trisomy 21, one pair). Of the remaining 150 eligible pairs, 32 pairs were monochorionic. A total of 639 children (317 pairs of twins and five singletons) underwent the *Bayley Scales of Infant and Toddler Development* between July 2010 and June 2012 (figure 6, page 82).

The mean gestational age of the entire group was 35.7 months. A breakdown of study participants according to group were as follows:

- Growth-discordant group: 238 children (119 pairs out of a possible 150 pairs, 79% follow-up rate).

- Control group: 222 children (111 pairs recruited out of 120 pairs, 92% follow-up rate). The control groups were chosen randomly and matched for gestational age at birth with each growth-discordant twin pair.

- High-risk group: 174 children (87 pairs). These children from the concordant group of twins were identified as “high risk” by the *ASQ-3*, thus warranting a more detailed developmental assessment.
• Singletons: 5 children (only 6 out of 25 completed the ASQ-3). The majority of these parents did not wish to participate in the NOTES Study.

Table 6 (page 83) illustrates the general demographics of the participating groups in phase 2 of the NOTES Study.
Figure 6: Flow diagram of growth-discordant twin pairs from ESPRiT and NOTES Study

159 growth-discordant twin pairs from ESPRiT Study

Single fetal demise ≥24 weeks: 1, single neonatal death: 3, childhood death: 1

154 growth-discordant pairs

4 twin pairs excluded: Trisomy 21: 1 Autism: 3

150 growth-discordant twin pairs approached for the NOTES Study

119 growth-discordant twin pairs recruited for the NOTES Study
Table 6: Demographics for participating twin pairs in the NOTES Study, arranged by groups

<table>
<thead>
<tr>
<th></th>
<th>GD¹ Pairs</th>
<th>Control pairs</th>
<th>High-risk pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pairs</td>
<td>119</td>
<td>111</td>
<td>87</td>
</tr>
<tr>
<td>Mean GA²</td>
<td>35.2 (+/-2.7)</td>
<td>35.4 (+/-2.6)</td>
<td>36.5 (+/-2.0)</td>
</tr>
<tr>
<td>Mean BW³ (grams)</td>
<td>2221 (+/-700)</td>
<td>2370 (+/-533)</td>
<td>2646 (+/-517)</td>
</tr>
<tr>
<td>MCDA⁴ pairs</td>
<td>24 (20%)</td>
<td>25 (23%)</td>
<td>9 (11%)</td>
</tr>
<tr>
<td>Elective LSCS⁵</td>
<td>70 (59%)</td>
<td>44 (40%)</td>
<td>36 (43%)</td>
</tr>
<tr>
<td>Emergency LSCS (pre-labour)</td>
<td>21 (18%)</td>
<td>11 (10%)</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>Assisted conception</td>
<td>37 (36%)</td>
<td>29 (27%)</td>
<td>12 (14%)</td>
</tr>
</tbody>
</table>

¹GD- growth discordant, ²GA- gestational age, ³BW- birth weight, ⁴MCDA- monochorionic, diamniotic, ⁵LSCS- lower-segment caesarean section, ⁶IVF- In-vitro fertilization, ⁷ICSI-Intracytoplasmic sperm injection

**INTER-RATER AGREEMENT**

A total of four professionals performed all Bayley Scales Assessments. An inter-rater reliability was performed using a linear mixed-effects model. No evidence of systemic differences in ratings for any item of the Bayley Scales was found. A maximum of only 1% of the total variability was due to examiner differences.
CHARACTERISTICS OF THE GROWTH-DISCORDANT TWIN PAIRS

Table 7 (page 85) is a detailed demographics table illustrating characteristics of both the growth-discordant group and control group of twins. Of the 119 pairs of growth-discordant twins recruited, 24 pairs were MC twins.

The mean gestational age on the day of developmental assessment (Bayley Scales of Infant and Toddler Development, 3rd Edition) was 35.2 months. Of note, only 15 pairs of growth-discordant twins were under 32 weeks, while 58 pairs were 36+0 weeks’ gestation or over.

The majority of the growth-discordant twin pairs were both appropriately grown (AGA) for their gestation at birth (73 pairs). Only two pairs comprised of twins where both were less than the 5th percentile. Forty-four pairs of twins comprised of one AGA twin (>5th percentile) and one small for gestational age (SGA) twin (<5th percentile).
Table 7: Demographics of growth-discordant twin pairs and control pairs

<table>
<thead>
<tr>
<th></th>
<th>GD pairs (N=119)</th>
<th>Control pairs (N=111)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age (Weeks)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)*</td>
<td>35.2(+/-2.7)</td>
<td>35.4(+/-2.6)</td>
<td>0.55</td>
</tr>
<tr>
<td>GA&lt;30</td>
<td>7 (6%)</td>
<td>6 (6%)</td>
<td></td>
</tr>
<tr>
<td>30≥GA&lt;32</td>
<td>8(7%)</td>
<td>5 (5%)</td>
<td></td>
</tr>
<tr>
<td>32≥GA&lt;34</td>
<td>13 (11%)</td>
<td>15 (14%)</td>
<td></td>
</tr>
<tr>
<td>34≥GA&lt;36</td>
<td>33 (28%)</td>
<td>27 (24%)</td>
<td></td>
</tr>
<tr>
<td>36≥GA&lt;37</td>
<td>22 (18%)</td>
<td>22(20%)</td>
<td></td>
</tr>
<tr>
<td>GA≥37</td>
<td>36 (30%)</td>
<td>36 (32%)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean birthweight (g) (SD)</strong></td>
<td>2221(+/-700)</td>
<td>2370(+/-533)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>MCDA</strong>¹⁰ pairs</td>
<td>24 (20%)</td>
<td>25 (23%)</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Gestational age mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCDA pairs</strong></td>
<td>33.6 (+/-3.1)</td>
<td>35.4(+/-1.8)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>DC</strong>¹¹ pairs</td>
<td>35.6(+/-1.8)</td>
<td>35.4(+/-2.8)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>AGA</strong>¹² pairs²</td>
<td>73 (61%)</td>
<td>106 (95%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>SGA</strong>¹³/AGA pairs³</td>
<td>44 (37%)</td>
<td>4 (4%)</td>
<td></td>
</tr>
<tr>
<td><strong>SGA pairs</strong></td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

1 Percentages for completed weeks of gestation are cumulative.

2 BW both twins above 5th centile. AGA: Appropriately grown for gestational age.

3 One twin BW above 5th centile, other twin BW below 5th centile. SGA: Small for gestational age.
<table>
<thead>
<tr>
<th></th>
<th>GD (n=130)</th>
<th>Control (n=100)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elective cesarean delivery</td>
<td>70 (59%)</td>
<td>44 (40%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Emergency pre-labour cesarean delivery</td>
<td>21 (18%)</td>
<td>11 (10%)</td>
<td></td>
</tr>
<tr>
<td>Assisted conception(^4)</td>
<td>37 (36.3%)</td>
<td>29 (27.1%)</td>
<td></td>
</tr>
<tr>
<td>Maternal age</td>
<td>33.5 (+/- 5.3)</td>
<td>33.0 (+/- 5.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>Maternal BMI(^{14})</td>
<td>25.7 (+/-4.5)</td>
<td>26.1 (+/-5.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>112 (94%)</td>
<td>105 (95%)</td>
<td>0.88</td>
</tr>
<tr>
<td>Social Class (Professional)(^5)</td>
<td>55 (54%)</td>
<td>69 (63%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Neonatal morbidity(^6)</td>
<td>17 (14%)</td>
<td>10 (9%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Age on day of Bayley Assessment in months (range)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight discordance at assessment (%)</td>
<td>10.1 (+/-5.8)</td>
<td>7.1 (+/- 5.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Weight discordance at assessment &gt; 20%</td>
<td>13 (11%)</td>
<td>4 (4%)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

\(^4\) Includes IVF and ICSI.

\(^5\) Social class was unobtainable for 9 (8%) of the GD group and 10 (9%) of control group. “Professional” was defined as managerial, professional, or technical (skilled).

\(^6\) Composite outcome of IVH, HIE, radiological evidence of PVL or NEC.

\(^7\) GD- growth discordant, SD- standard deviation, GA- gestational age, MCDA- monochorionic, diamniotic. DC- dichorionic, AGA- appropriately grown for gestational age, SGA- small for gestational age, BMI- body mass index
GROWTH-DISCORDANT TWIN PAIRS AND DEGREE OF DISCORDANCE

The majority of the 119 growth-discordant twin pairs had a birthweight growth discordance of 20% to 29%. Eighty pairs (67%) of the cohort fell into this group. There were 32 pairs (27%) that had a birthweight discordance of 30% to 39%. Only 7 pairs (6%) had a birthweight discordance of 40% or more.

Figure 7 (page 88) shows the distribution of twin-pair birth weight (BW) discordance, presenting the number of twin pairs (twin-pair index) in the NOTES Study on the x axis according to BW discordance. The peak at 20% discordance represents the cut-off to the study groups (discordant vs concordant). The concordant twins (<20%) were sampled for the larger ESPRiT Study population. The generally smooth appearance shows good representation of all discordance levels within the study cohort.
PHYSICAL GROWTH IN THE GROWTH-DISCORDANT TWIN GROUP: INTER-TWIN RESULTS

As part of phase two of the NOTES Study, each twin had their height, weight, and head circumference recorded. This was done on the day of assessment with the Bayley Scales. When comparing physical growth between the smaller and larger twin in growth discordant twin pairs in the first two to three years of life, we found the following:

- Height: The smaller twin at birth remained significantly shorter than his/her larger co-twin at 2 to 3 years of age with a mean height of 93.0 cm +/- 4.7 versus a mean height of 95.3 cm +/- 4.9 for the
larger co-twin (p <0.001). Similar results were obtained for both weight and head circumference (table 8, page 89, figure 8, page 90).

Table 8: Physical growth parameter results in the growth discordant twin pair, inter-twin analysis: Larger twin versus smaller twin

<table>
<thead>
<tr>
<th>Score/Covariate</th>
<th>Twin size N=119</th>
<th>Mean</th>
<th>SD¹</th>
<th>Min, Max</th>
<th>Difference in mean scores 95% CI²</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>Large 95.3</td>
<td>93.0</td>
<td>4.9</td>
<td>84, 109</td>
<td>2.3 (2.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>95.3</td>
<td>4.7</td>
<td>73, 105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Large 15.2</td>
<td>14.0</td>
<td>2.4</td>
<td>10.3, 25</td>
<td>1.2 (7.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>15.2</td>
<td>2.0</td>
<td>9.4, 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head Circumference (cm)</td>
<td>Large 50.5</td>
<td>49.6</td>
<td>1.8</td>
<td>46, 57</td>
<td>0.9 (1.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>50.5</td>
<td>1.6</td>
<td>43, 57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Paired t-test

¹SD- standard deviation, ²CI- confidence interval
Figure 8: Box plots for Bayley Scales results in the growth-discordant twin pairs.

(Paired t-test)

Larger twin versus smaller twin: Head circumference, weight, and height on day of assessment. Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers).
We also assessed the degree of catch-up growth which occurred between birth and the developmental assessment in the smaller twin in GD pairs. Figures 9 (page 92) and 10 (page 93) compare the Z-score for weight and head circumference (at birth and on day of *Bayley Scales Assessment*) between the smaller and larger twin in the growth-discordant twin pairs.

At birth, the smaller twin is on average 1.32 SD below the mean for weight while the larger twin is on average 0.33 SD above the mean. On the day of the *Bayley Scales Assessment* (labelled assessment above figure), the smaller twin is on average 0.16 SD above the mean for weight while the larger twin is on average 0.83 SD above the mean. The difference in SD between the smaller and larger twin at birth was 1.65, the difference in SD between the smaller and larger twin on the day of assessment was 0.67 (p< 0.001).

The head circumference of the smaller twin is 0.74 SD below the mean while the larger twin is on average 0.52 SD above the mean at birth. On the day of the *Bayley Scales Assessment*, the smaller twin is on average 0.6 SD above the mean and the larger twin is on average 1.33 SD above the mean. The difference in SD between the larger and smaller twin at birth was 1.26 vs 0.73 on the day of assessment (p< 0.001).

In summary, the smaller twin’s weight and head circumference remains smaller at two to three years of life compared to the larger co-twin, although significant catch-up growth is exhibited. It is reassuring that
the mean Z-score for head circumference and weight of the smaller twin is greater than 0 (i.e., above average) on the day of assessment.

Figure 9: Boxplots illustrating Z-scores for weight at birth and weight on day of the Bayley Scales Assessment in growth-discordant twin pairs: Smaller twin versus larger twin.

*p-value < 0.001

Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers).
Figure 10: Boxplots illustrating Z-scores for head circumference at birth and on day of the Bayley Scales Assessment in growth-discordant twin pairs: Smaller twin versus larger twin.

p-value <0.001

Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers).
NEURODEVELOPMENTAL OUTCOME OF GROWTH-DISCORDANT TWINS: INTER-TWIN RESULTS

The main analysis in phase two of the NOTES Study involved inter-twin analysis within the growth-discordant group of twins as well as comparing developmental outcomes between the growth-discordant group and the control group of twins.

Composite and scaled scores from the Bayley Scales of Infant and Toddler Development, 3rd Edition, in the areas of cognition, language, and motor skills were compared between the smaller and larger twin in each growth-discordant pair. To account for correlations within twin pairs, a paired t-test was used for inter-twin comparisons.

When comparing the results of the Bayley Scales, 3rd Edition, between the smaller and larger twin, the smaller twin had significantly lower scores in several areas of development:

- **Cognitive Scale:** The mean composite score of the smaller twin in this category was 100.9 +/-9.2 vs for the larger twin was 102.6+/-9.7 (mean difference -1.7, 95% confidence interval = -3.1 to -0.3). This was statistically significant with a p-value of 0.02. (Table 9, page 96, figure 11, page 97).

Similar significant results were also obtained in language composite and motor composite scores as well as receptive language and fine motor scaled scores (table 9, page 96, figure 11 to 13, pages 97-99).
Figures 11 to 13 illustrate boxplots showing results of the *Bayley Scales* between the smaller and larger twin in the growth-discordant pairs. Boxplots represent 25\(^{\text{th}}\) and 75\(^{\text{th}}\) centiles (box), 50\(^{\text{th}}\) centile (box internal line), and 10\(^{\text{th}}\) and 90\(^{\text{th}}\) centiles (extent of whiskers).

In summary, when comparing the smaller twin to the larger twin in the growth-discordant twin pairs, the smaller twin performed significantly worse in the developmental categories of cognition, receptive communication, composite language, fine motor, and composite motor categories.
Table 9: Bayley Scales results in the growth-discordant twin pairs, inter-twin analysis: Larger twin versus smaller twin

<table>
<thead>
<tr>
<th>Score/covariate</th>
<th>Twin size</th>
<th>Mean</th>
<th>SD¹</th>
<th>Min, Max</th>
<th>Difference in mean scores</th>
<th>95% CI</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=119</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive Composite Score</td>
<td>Large</td>
<td>102.6</td>
<td>9.7</td>
<td>80, 135</td>
<td>-1.7(-3.1, -0.3)</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>100.9</td>
<td>9.2</td>
<td>80,140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language Composite Score</td>
<td>Large</td>
<td>106.9</td>
<td>10.8</td>
<td>77, 135</td>
<td>-1.8(-3.3, -0.3)</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>105.1</td>
<td>10.4</td>
<td>79, 127</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressive Communication Scaled Score</td>
<td>Large</td>
<td>11.0</td>
<td>2.0</td>
<td>6, 16</td>
<td>-0.2(-0.5, 0.09)</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>10.8</td>
<td>2.1</td>
<td>5, 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptive Communication Scaled Score</td>
<td>Large</td>
<td>11.3</td>
<td>2.0</td>
<td>5, 16</td>
<td>-0.3(-0.6, -0.1)</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>11.0</td>
<td>1.7</td>
<td>7, 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Composite Score</td>
<td>Large</td>
<td>106.7</td>
<td>11.1</td>
<td>82, 142</td>
<td>-2.2(-4.0, -0.4)</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>104.5</td>
<td>10.0</td>
<td>82, 136</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine Motor Scaled Score</td>
<td>Large</td>
<td>11.0</td>
<td>2.3</td>
<td>6, 18</td>
<td>-0.4(-0.8, -0.1)</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>10.6</td>
<td>1.9</td>
<td>7, 18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross Motor Scaled Score</td>
<td>Large</td>
<td>12.0</td>
<td>10.5</td>
<td>6, 17</td>
<td>-0.3(-0.7, -0.1)</td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>11.7</td>
<td>2.3</td>
<td>4, 17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Paired t-test

¹SD- standard deviation, ²CI- confidence intervals
Figure 11: Boxplots for Bayley Scales results in the growth-discordant twin pairs. Smaller twin versus larger twin: Cognitive, language, and motor composite scores (paired t-test).

Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers).
Figure 12: Boxplots for Bayley Scales results in the growth-discordant twin pairs. Smaller twin versus larger twin: Expressive and receptive language scaled scores (paired t-test).
Figure 13: Boxplots for Bayley Scales results in the growth-discordant twin pairs. Smaller twin versus larger twin: Fine and gross motor scaled scores (paired t-test).

Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers).
NEURODEVELOPMENTAL OUTCOME OF GROWTH-DISCORDANT TWINS COMPARED WITH CONCORDANT GROWTH CONTROL GROUP: INTER-TWIN RESULTS

Figure 14 (page 101) illustrates the mean differences in Bayley Scales scores between larger and smaller twins (larger minus smaller) in the growth-discordant and concordant (control) group of twins. The comparison is between twins in each pair and not between the concordant and discordant. Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers). There are no significant differences between twins in the concordant group.

GROWTH-DISCORDANT TWIN PAIRS AND BEHAVIOUR: INTER-TWIN RESULTS

There was no statistical difference in any area of behaviour of the social-emotional or adaptive behaviour scales nor was there any difference in internalizing and externalizing behaviours as identified through the Child Behaviour Checklist when comparing the smaller twin to the larger twin in our growth-discordant twin pairs (table 10, page 102).
Figure 14: Inter-twin mean differences in Bayley Scores for discordant and concordant twin pairs (paired t-test).

Boxplots represent 25th and 75th centile (box), 50th centile (box internal line), and 10th and 90th centiles (extent of whiskers).
Table 10: Behavioural characteristics results in the growth discordant twin pairs, inter–twin analysis: Larger twin versus smaller twin.

<table>
<thead>
<tr>
<th>Score/Covariate</th>
<th>Twin size N=119</th>
<th>Mean</th>
<th>SD(^1)</th>
<th>Min, Max</th>
<th>Difference in mean score</th>
<th>p-value(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social-Emotional Scale</td>
<td>Large</td>
<td>110.0</td>
<td>17.4</td>
<td>65, 140</td>
<td>0.3</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>109.7</td>
<td>17.1</td>
<td>65, 140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adaptive Behaviour Scale</td>
<td>Large</td>
<td>99.4</td>
<td>12.4</td>
<td>67, 134</td>
<td>0.5</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>98.9</td>
<td>12.6</td>
<td>47, 127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC(^2)-Internalising Behaviour</td>
<td>Large</td>
<td>0.7</td>
<td>0.6</td>
<td>2, 0</td>
<td>0.1</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>0.8</td>
<td>0.8</td>
<td>2, 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC-Externalising Behaviour</td>
<td>Large</td>
<td>0.64</td>
<td>0.4</td>
<td>2, 0</td>
<td>0.06</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>0.7</td>
<td>0.6</td>
<td>2, 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) Paired t-test
\(^1\) SD- standard deviation, CBC-child behaviour checklist

SUB-GROUP ANALYSIS OF FACTORS CONTRIBUTING TO NEURODEVELOPMENTAL OUTCOME IN GROWTH-DISCORDANT TWINS

As established in chapter 2, twins are at higher risk for perinatal mortality and morbidity compared to singletons (Hack 2008). In addition to growth discordance, other factors that may contribute to adverse developmental outcomes include:

- Intrauterine growth restriction (IUGR)/small for gestational age (SGA) and degree of discordance
• Chorionicity
• Prematurity (discussed later in this chapter)

The primary purpose of the NOTES Study was to demonstrate that growth discordance is an independent risk factor for adverse developmental outcomes. To accomplish this, the additional risk factors stated above were examined in the growth-discordant twin cohort.

**IUGR/SGA**

The majority (73 pairs) of the growth-discordant twins in the NOTES Study were both appropriately grown for gestational age (AGA, both >5th percentile).

In order to remove SGA birthweights as a potential confounding factor for adverse neurodevelopmental outcomes, the *Bayley Scales* results of the 73 AGA growth discordant pairs were analysed.

Cognitive Category: Despite both twins having birthweights greater than the 5th percentile, the smaller twin still performed significantly worse in the cognitive category compared to his/her larger co-twin (100.6+/-8.0 vs.102.5+/-9.4 p-value = 0.02). Similar statistically significant results were obtained in the language and motor scales (table 11, page 104).
As stated at the start of chapter 4, only 39 pairs of twins comprised of 1 SGA (<5th percentile) twin and 1 AGA (>5th percentile) twin. No statistical difference was found in cognitive, language, or motor outcome between SGA and AGA twins, probably due to the small sample size (table 12, page 105).

Table 11: Bayley Scales results of appropriately grown (AGA>5th percentile) growth-discordant twin pairs, inter-twin analysis: Larger twin versus smaller twin.

<table>
<thead>
<tr>
<th>Score / covariate</th>
<th>Twin size</th>
<th>Mean</th>
<th>SD¹</th>
<th>Min, Max</th>
<th>Difference in mean scores</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive Composite Score</td>
<td>Large</td>
<td>102.5</td>
<td>9.4</td>
<td>80, 130</td>
<td>1.9</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>100.6</td>
<td>8.0</td>
<td>85, 125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language Composite Score</td>
<td>Large</td>
<td>106.6</td>
<td>11.3</td>
<td>77, 135</td>
<td>2.1</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>104.5</td>
<td>10.8</td>
<td>79, 124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Composite Score</td>
<td>Large</td>
<td>106.7</td>
<td>11.4</td>
<td>82, 142</td>
<td>2.1</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>104.6</td>
<td>10.8</td>
<td>82, 136</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Paired t-test

¹SD- standard deviation
Table 12: Bayley Scales results of growth-discordant (AGA > 5th percentile/SGA < 5th percentile) twin pairs, inter-twin analysis: Larger twin versus smaller twin.

<table>
<thead>
<tr>
<th>Score/covariate</th>
<th>Twin size</th>
<th>Mean</th>
<th>SD¹</th>
<th>Min, Max</th>
<th>Difference in mean scores</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Composite</td>
<td>Large</td>
<td>101.9</td>
<td>10.3</td>
<td>85, 135</td>
<td>0.6</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>101.3</td>
<td>12.4</td>
<td>80, 140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language Composite</td>
<td>Large</td>
<td>106.9</td>
<td>8.4</td>
<td>86, 127</td>
<td>0.3</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>106.6</td>
<td>9.3</td>
<td>91, 127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Composite</td>
<td>Large</td>
<td>105.3</td>
<td>10.4</td>
<td>88, 124</td>
<td>1.2</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>104.1</td>
<td>7.7</td>
<td>85, 118</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Paired t-test

¹SD- standard deviation

Chorionicity

In the NOTES Study, 24 growth-discordant twin pairs were monochorionic twins; this was 20% of our recruited growth-discordant twin cohort. These 24 pairs were compared to the 95 remaining dichorionic twin pairs in the growth-discordant cohort. Monochorionic twins had significantly lower scores in the areas of receptive and expressive communication, language composite, and on the gross motor and composite motor scales after adjusting for gestational age at delivery and incorporating inter-twin correlation. No statistical significance was found in the social-emotional scale or adaptive behaviour characteristics between the 2 groups.
Table 13 (page 106) illustrates these findings. Inter-twin analysis within the monochorionic growth discordant group of twins was attempted; however, a small sample size (24 pairs) precluded analysis of significant differences between the smaller and larger twin in developmental outcomes.

**Table 13: Bayley Scales results for growth-discordant monochorionic twins vs growth-discordant dichorionic twins.**

<table>
<thead>
<tr>
<th>Category / Covariate</th>
<th>Chorionicity</th>
<th>Mean</th>
<th>SD¹</th>
<th>Difference in mean scores</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dichorionic: n=95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive Composite Score</td>
<td>Monochorionic: n=24</td>
<td>102.0</td>
<td>0.7</td>
<td>1.3</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.7</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language Composite Score</td>
<td></td>
<td>106.7</td>
<td>0.8</td>
<td>3.7</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>103.0</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressive Language Scaled Score</td>
<td></td>
<td>11.0</td>
<td>0.1</td>
<td>0.8</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.2</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptive Language Scaled Score</td>
<td></td>
<td>11.3</td>
<td>0.1</td>
<td>0.5</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.8</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Composite Score</td>
<td></td>
<td>106.6</td>
<td>0.8</td>
<td>5.2</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101.4</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine Motor Scaled Score</td>
<td></td>
<td>11.0</td>
<td>0.2</td>
<td>0.6</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.4</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross Motor Scaled Score</td>
<td></td>
<td>11.2</td>
<td>0.2</td>
<td>1.2</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social Emotional Composite Score</td>
<td></td>
<td>110.9</td>
<td>1.2</td>
<td>3.6</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>107.3</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mixed multivariate analysis model adjusting for gestational age at delivery and incorporating inter-twin correlations.

1SD- standard deviation, 2GAC- general adaptive composite (score)

**GROWTH-DISCORDANT TWIN PAIRS VERSUS CONTROL GROUP**

In addition to inter-twin analysis of growth-discordant twins, the growth-discordant twin pairs were also compared to a control group comprising of concordant twin pairs from the study cohort matched for gestation with our growth-discordant twin pairs.

The growth-discordant twin pairs were compared to the control group in terms of neurodevelopmental outcome and behavioural issues. The average scores of the growth-discordant pairs and control pairs were analysed as well as comparing the small twin in the growth-discordant group to the control group.

When compared to the control group, the growth-discordant twin pairs did not perform significantly worse in any developmental or behavioural parameter nor was there any difference between the control group and the smaller twin in the growth-discordant twin pairs. We controlled for social class when analysing differences between the
growth-discordant group of twins and the control group of twins and there was still no significant difference between the groups. Table 14 (page 108) illustrates these findings.

Table 14: Bayley Scales composite scores and CBC scores: Control group versus growth-discordant group of twins.

<table>
<thead>
<tr>
<th>Score</th>
<th>Twin Groups: Control/Growth Discordant (Average Scores)</th>
<th>Number</th>
<th>Mean Score</th>
<th>SD¹</th>
<th>Min- Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Composite Score</td>
<td>Control GD²- small twin GD</td>
<td>222</td>
<td>100.4</td>
<td>7.2</td>
<td>85, 123</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>119</td>
<td>100.9</td>
<td>9.2</td>
<td>80, 140</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>238</td>
<td>101.8</td>
<td>9.5</td>
<td>80, 140</td>
<td></td>
</tr>
<tr>
<td>Language Composite Score</td>
<td>Control GD-small twin GD</td>
<td>222</td>
<td>104.0</td>
<td>10.1</td>
<td>58, 125</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>119</td>
<td>105.1</td>
<td>10.4</td>
<td>79, 127</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>238</td>
<td>106.1</td>
<td>10.7</td>
<td>77, 135</td>
<td></td>
</tr>
<tr>
<td>Motor Composite Score</td>
<td>Control GD- small twin GD</td>
<td>222</td>
<td>104.7</td>
<td>10.6</td>
<td>76, 150</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Control GD- small twin GD</td>
<td>119</td>
<td>104.5</td>
<td>10.0</td>
<td>82, 136</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Control GD- small twin GD</td>
<td>238</td>
<td>105.6</td>
<td>10.6</td>
<td>82, 136</td>
<td></td>
</tr>
<tr>
<td>Social Emotional Composite Score</td>
<td>Control GD-small twin GD</td>
<td>222</td>
<td>110.3</td>
<td>17.3</td>
<td>68, 140</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>119</td>
<td>109.7</td>
<td>17.1</td>
<td>65, 140</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>238</td>
<td>109.9</td>
<td>17.2</td>
<td>65, 140</td>
<td></td>
</tr>
<tr>
<td>Adaptive Behaviour GAC³</td>
<td>Control GD-small twin GD</td>
<td>222</td>
<td>99.3</td>
<td>12.2</td>
<td>65, 138</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>119</td>
<td>98.9</td>
<td>12.6</td>
<td>47, 127</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Control GD-small twin GD</td>
<td>238</td>
<td>99.0</td>
<td>12.5</td>
<td>47, 134</td>
<td></td>
</tr>
</tbody>
</table>
Mixed multivariate analysis model adjusting for gestational age at delivery, social class, and incorporating inter-twin correlation.

1SD-standard deviation, 2GD-growth discordant, 3GAC-general adaptive composite (score), 4CBC-child behaviour checklist

**GROWTH-DISCORDANT TWIN PAIRS VERSUS HIGH-RISK GROUP**

174 children (87 pairs of twins) were identified as high risk based on ASQ scores. Two children in the high-risk group had profound global developmental delays of unknown cause. Two other children had been diagnosed with CP (both mobile, one with walking aid). Another six scored a 7 in two or more areas of development and another thirteen scored a 7 in one area of development. The remaining 133 children scored in either the normal or low-normal range in the Bayley Scales Assessment. Not surprisingly, when compared with the growth-discordant group of twins, the high risk performed significantly worse in all areas of development (p-value <0.001 in all areas of development) (table 15, page 111).
Table 15: Bayley Scales results for growth-discordant twin pairs vs high-risk group of twins

<table>
<thead>
<tr>
<th>Category / Covariate</th>
<th>Group</th>
<th>Mean</th>
<th>SD³</th>
<th>Difference in mean scores</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GD¹: n=119</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive Composite Score</td>
<td>GD</td>
<td>101.8</td>
<td>0.85</td>
<td>4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>96.9</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language Composite Score</td>
<td>GD</td>
<td>106.1</td>
<td>1.09</td>
<td>10.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>95.3</td>
<td>1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressive Language</td>
<td>GD</td>
<td>10.9</td>
<td>0.21</td>
<td>1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Scaled Score</td>
<td>HR</td>
<td>9.1</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptive Language</td>
<td>GD</td>
<td>11.2</td>
<td>0.18</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Scaled Score</td>
<td>HR</td>
<td>9.6</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Composite Score</td>
<td>GD</td>
<td>105.6</td>
<td>0.99</td>
<td>7.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>98.2</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine Motor Scaled Score</td>
<td>GD</td>
<td>10.9</td>
<td>0.18</td>
<td>1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>9.9</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross Motor Scaled Score</td>
<td>GD</td>
<td>10.9</td>
<td>0.18</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>9.6</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social Emotional</td>
<td>GD</td>
<td>110.3</td>
<td>1.50</td>
<td>12.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Composite Score</td>
<td>HR</td>
<td>97.4</td>
<td>1.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adaptive Behaviour GAC⁴</td>
<td>GD</td>
<td>99.0</td>
<td>1.12</td>
<td>13.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>85.2</td>
<td>1.42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mixed multivariate analysis model adjusting for gestational age at delivery and incorporating inter-twin correlations.

¹GD- growth discordant, ²HR- high risk, ³SD- standard deviation, GAC- general adaptive composite (score)
PREMATURITY

The majority of the growth-discordant twins in the NOTES Study were term or near-term twins; 45% or 54 pairs were born at 36 weeks or over. Fifteen pairs (13%) were born at under 32 weeks’ gestation; only seven (6%) pairs were born at less than 30 weeks’ gestation. Because it is well established that prematurity has a negative impact on neurodevelopmental outcome, we analysed the impact of growth discordance and prematurity in an attempt to establish which has a greater impact on cognitive outcome.

After controlling for birthweight by multiple regression analysis, prematurity had a far greater negative impact on cognitive performance than growth discordance did when born prior to 33 weeks’ gestation (≤32+6). At 33+0 weeks’ gestation, both prematurity and growth discordance had a significant negative impact on cognitive outcome. By 34+0 weeks’ gestation, only the effect of growth discordance remained significant. These findings are illustrated in table 16 (page 113). The left side of the table compares composite cognitive scores of infants above and below the gestational age cut-off while the right-hand columns show scores for the larger and smaller twins of the group below the gestational age cut-off. Figure 15 (page 114) represents the same data and further illustrates the effect of growth discordance on cognitive outcome between the smaller and larger twins to be small but relatively constant as gestation increases (dashed lines), while the effect
of gestation (solid lines) below 33 weeks has a far greater negative impact on cognitive outcomes (Halling 2016).

Table 16: Composite Cognitive Scores in the growth-discordant group of twins in relation to GA cut offs and birthweight discordance.

<table>
<thead>
<tr>
<th>GA¹ Cutoff</th>
<th>Completed weeks of gestation at delivery</th>
<th>Birthweight Discordance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Score</td>
<td>Mean Difference/ p-value</td>
</tr>
<tr>
<td></td>
<td>GA &lt; cutoff</td>
<td>GA ≥ cutoff</td>
</tr>
<tr>
<td>30 weeks (n=7)</td>
<td>94.3</td>
<td>102.3</td>
</tr>
<tr>
<td>31 weeks (n=11)</td>
<td>95.4</td>
<td>102.4</td>
</tr>
<tr>
<td>32 weeks (n=15)</td>
<td>96.7</td>
<td>102.5</td>
</tr>
<tr>
<td>33 weeks (n=21)</td>
<td>98.1</td>
<td>102.6</td>
</tr>
<tr>
<td>34 weeks (n=28)</td>
<td>99.7</td>
<td>102.4</td>
</tr>
<tr>
<td>35 weeks (n=46)</td>
<td>100.2</td>
<td>102.8</td>
</tr>
<tr>
<td>36 weeks (n=61)</td>
<td>100.2</td>
<td>103.4</td>
</tr>
<tr>
<td>37 weeks (n=83)</td>
<td>101.2</td>
<td>103.2</td>
</tr>
</tbody>
</table>

Multivariate Analysis of Composite Cognitive Score in Growth-Discordant Twins (N=119 Pairs) adjusting for gestational age at delivery and incorporating inter-twin correlations (mixed model). Categories are cumulative for gestational age at delivery < cut-off.

¹GA- gestational age
Figure 15: Effect of prematurity on composite cognitive score in growth-discordant twins. (N=119 pairs) adjusted for gestational age at delivery. 

Multiple regression analysis of composite cognitive score with growth discordance and gestational age at delivery as predictors. Numbers are cumulative for gestational age at delivery < cut offs, values shown are adjusted means. **p≤0.01, *p≤0.05
CHAPTER 4: DISCUSSION
THE NOTES STUDY

The NOTES Study represents one of the largest studies undertaken to investigate neurodevelopmental outcome of growth-discordant twins. The strengths of this study lie in its numbers and high follow-up rate of the growth-discordant twin pairs (79%). With over 100 growth-discordant twin pairs participating, this study is uniquely placed in allowing examination of growth discordance as an independent risk factor for adverse developmental outcomes as well as examining other factors that may contribute such as chorionicity, IUGR/SGA, and prematurity. Quantifying the risk of growth discordance on cognitive outcome was also compared to the effects of prematurity.

BAYLEY SCALES AND INTER-RATER VARIABILITY

The NOTES Study took place across 8 centres around the Republic of Ireland and Northern Ireland. Due to the distance between centres, 4 examiners were involved in carrying out the assessments.

The Bayley Scales of Infant and Toddler Development is a standardised developmental assessment. Training is required in order to become qualified at carrying out assessments. Items are demonstrated in very exact ways and a child must meet certain criteria in order to pass an item. Because of this, the results are reproducible between qualified examiners.
As part of the NOTES Study, an inter-rater reliability check was performed and found no evidence of systemic differences in ratings for all items on the Bayley Scales; a maximum 1% of the total variability was due to examiner differences.

SGA TWINS AND DEGREE OF DISCORDANCE

Studies suggest that IUGR/SGA have a negative effect on long-term neurodevelopmental outcomes (Chaudhari 1997, Shah 2011). This was discussed in detail in chapter 1. For obvious reasons, growth-discordant twins are at particularly high risk of having birth weights which are small for gestational age (BW less than 5th percentile). Interestingly, the ESPRiT Study concluded that a birthweight consistent with SGA less than the 5th percentile was associated with a much lower risk of adverse perinatal outcome than 18% birth weight discordance among dichorionic twins (Breathnach 2011).

The NOTES Study did not demonstrate adverse neurodevelopmental outcomes in those growth-discordant twins where 1 twin was SGA and the other was AGA. Despite being growth discordant, the majority of the twins in the NOTES Study were both AGA at birth (73 pairs). Only 39 pairs consisted of one AGA twin and one SGA twin. The results of analysis in this group did not yield any significant findings. Either the effects of IUGR/SGA did not have a negative impact on developmental outcomes of growth-discordant twins in our cohort or the sample size was too small to reach statistical significance.
The cut-off for significant growth discordance in the NOTES Study was 20% (as initially defined by the ESPRiT Study). The NOTES Study found no additional negative consequences on developmental outcome when varying degrees of significant growth discordance was looked at. Once again, this is possibly due to the fact that the majority (80 pairs, 67%) of our twin cohort had a birthweight growth discordance between 20% to 29%, while 27% (32 pairs) had a birthweight growth discordance of 30% to 39%. Extreme growth discordance of 40% or more was only found in 6% (7 pairs) of our cohort.

**PHYSICAL GROWTH IN THE GROWTH-DISCORANT TWIN GROUP**

The NOTES Study showed that although the smaller twin demonstrated good catch-up growth, he/she remained smaller (height, weight, and head circumference) compared to the larger twin at two to three years of age. Interestingly, there is evidence that the smaller twin in growth discordant twin pairs remains smaller throughout early childhood and into school age (Monset-Couchard 2004, Boghossian 2019, Swami 2018) and can persist into adolescence (Schulte 2016) and even adulthood (Loos 2002). Several studies found that the majority of the “catch-up growth” in the smaller twin occurred in the first two to three years of life (Schulte 2016, Monset-Couchard 2004). This was seen in both monochorionic and dichorionic twins when the smaller twin was SGA (BW less than 10th percentile) (Monset-Couchard 2004, Schulte
In 2019, Boghossian reported that the smaller twin in twin pairs with greater than 30% growth discordance remained lighter and had smaller head circumferences at 18 to 22 months compared to his/her larger co-twin (Boghossian 2019). Swami et al. reported similar findings at school age with the smaller twin still significantly lighter, shorter, and with a smaller head circumference compared to his/her larger co-twin (Swami 2018). In 2002, Loos et al. reported that birthweight growth discordance of greater than 15% between female twins (monochorionic and dichorionic) resulted in lower adult weight and height in the smaller twin. Interestingly, Schulte et al. (2016) reported an earlier start and fast progression of puberty in the smaller twin in female monochorionic twin pairs further contributing to shorter adult height.

**CHORIONICITY**

Evidence suggests that monochorionic twin pregnancies are at increased risk of adverse perinatal outcomes (Selman 2011, Hack 2008) when compared to dichorionic twin pregnancies.

Unique to monochorionic twin pregnancies, TTTS is associated with increased risks of both perinatal mortality and morbidity in addition to adverse neurodevelopmental outcomes when compared to uncomplicated MC and DC twin pregnancies (Selman 2011). This was discussed in detail in chapter 1. Even without TTTS, studies such as
Hack (2009) have demonstrated adverse neurodevelopmental outcomes in MC twins compared to DC twins.

The ESPRiT Study in 2011 also concluded that absolute morbidity risks (mortality, HIE, PVL, NEC, respiratory distress, or sepsis) were higher among discordant monochorionic twins when compared with discordant dichorionic twins at every level of birthweight discordance (Breathnach 2011). The NOTES Study results supports that this negative effect persists and impacts on later neurodevelopmental outcomes.

The NOTES Study showed that chorionicity plays a significant modifying role in later neurodevelopmental outcomes when the monochorionic growth-discordant twins were compared to the dichorionic growth-discordant twins.

Inter-twin analysis within the monochorionic growth-discordant twin group was not feasible due to small sample size (24 pairs). In 2019, however, a systemic review by Groene suggested that the smaller twin in growth-discordant monochorionic twin pairs is at increased risk for neurodevelopmental impairment compared to the larger co-twin. Swami et al. reported similar findings at school age (Groene 2019 and Swamy 2018).
SINGLE FETAL DEMISE

As discussed in chapter 1, single fetal death is not uncommon in twin pregnancies (Mahoney 2011), and when it occurs in the second and third trimesters, it can have devastating consequences on later neurodevelopment for the surviving twin (Woo 2000). Because of this, survivors of twin pairs from late single fetal demise were included (11 pairs) in the NOTES Study. The surviving co-twins of early neonatal deaths were also included for ethical reasons (12 pairs). The majority of these, however, did not wish to participate in the NOTES Study and only five underwent a Bayley Scales Assessment. Unfortunately, this sample was too small for analysis.

GROWTH DISCORDANCE AND DEVELOPMENT

Previous studies investigating growth discordance and development have mostly been small and have demonstrated a negative impact on only one or two areas of neurodevelopment (Goyen 2003, Ross 2012). More recently, similar findings have been found in larger studies (Swami 2018). The NOTES Study demonstrated growth discordance as a negative impact on the smaller twin across all three domains of development: cognition, language, and motor. The study also found that the smaller twin at birth remains smaller in all three parameters of physical growth (weight, height, and head circumference) at two to three years of age.
The main goal of the NOTES Study was to investigate whether growth discordance is an independent risk factor for adverse neurodevelopmental outcomes. The majority of our growth-discordant pairs were near term and appropriately grown for gestational age. After accounting for factors such as chorionicity, IUGR/SGA and prematurity, we established that growth discordance independently influences neurodevelopmental outcome.

Statistically, the smaller twin performed significantly worse than the larger twin in all three areas of development: cognition, language, and motor. However, the difference in the composite scores between larger and smaller twin was only a few points. Clinically, this may be of little importance. The majority of the smaller twins scored within normal limits in the Bayley Scales Assessment. Very few suffered developmental delay; only four small twins suffered from moderate delay while two larger twins suffered moderate developmental delay. No twins suffered severe delays. There were no cases of cerebral palsy in the growth-discordant twins where both twins survived. One twin (smaller twin) suffered from bilateral hearing loss requiring hearing aids. Using the EPICure criteria for developmental delay, the NOTES Study was unable to demonstrate higher rates of mild, moderate, or severe developmental delay in the smaller twin compared to the larger twin in growth-discordant twin pairs.
The *Bayley Scales, 3rd Edition*, is a recognised tool for detecting developmental delays in young children. However, its ability to do so with the same sensitivity as the *Bayley Scales, 2nd Edition*, has come under scrutiny. In 2011, for example, Moore found that in extremely preterm children at 29 to 41 months’ corrected age with composite scores under 70, the combined Bayley Score was 7 points higher in the 3rd Edition compared to the MDI score in the 2nd Edition. This gives the *Bayley Scales, 3rd Edition*, a sensitivity of only 58% and a specificity of 97% when compared to the 2nd Edition (Moore 2011). However, in composite scores under 80, *The Bayley Scales, 3rd Edition*, was found to have a sensitivity of 89% and a specificity of 99% (Moore 2011). Other studies have corroborated the finding that the *Bayley Scales, 3rd Edition*, possibly overestimates cognitive outcomes (Marlow 2005, Acton 2011, Vohr 2012); therefore, some subjects in our study may have more significant cognitive delays at school age. Edmonds (2010) demonstrated that the smaller twin had a progressively lower verbal intelligence quotient throughout childhood as the degree of growth discrepancy increased. It is likely that the differences observed in cognition, language, and motor scores in our study will continue to manifest to some degree as small but significant differences in intelligence quotients in later life.
The negative impact extreme prematurity has on later neurodevelopmental outcome has been well established through studies such as the EPICure Study (Marlow 2005). In addition, studies (Morse 2009, and Chyi 2008) have also demonstrated a negative impact on neurodevelopmental outcome of those born at a late preterm gestation when compared to term infants.

The impact of in-utero growth discordance was compared to the effect of prematurity on cognitive outcome of the growth-discordant group of twins. Although small, growth discordance had a consistent negative effect on cognitive outcome across varying gestational ages. The impact of prematurity on cognitive scores at less than 33 weeks’ gestation, however, was greater than the effect of growth discordance. At 33 weeks, both growth discordance and prematurity had a significant negative impact on cognitive scores. By 34 weeks’ gestation, birthweight discordance influenced cognitive outcome to a greater extent than did gestational age at birth (Halling 2016). This is an important finding which may influence the timing of delivery in twin pregnancies where significant growth discordance is observed.
OPTIMUM TIMING OF DELIVERY

In the past, it has been proposed that fetal lung maturity occurs earlier in twins than in singletons (Bakr 2006). This has possibly been used to help justify the delivery of even uncomplicated twins before term. Studies, however, have suggested that respiratory distress is one of the most common perinatal morbidities found in late preterm twins (Breathnach 2011, Qui 2008).

In the ESPRiT cohort, only 18% of term (37+ weeks’ gestation) twin pregnancies went into spontaneous labour. This cohort was used to establish the optimum timing for planned delivery of uncomplicated monochorionic and dichorionic pregnancies. Of the uncomplicated monochorionic twins in the ESPRiT Study, 17.5% were electively delivered prior to 37 weeks’ gestation with 2.3% being delivered electively at 34 weeks’ gestation. However, in this cohort, the risk of a composite measure of perinatal morbidity fell from 41% at elective LSCS at 34 weeks to 5% at 37 weeks (p-value <0.001). This must be balanced against the 1.5% risk of in-utero death in uncomplicated monochorionic twin pregnancies after 34 weeks’ gestation (Breathnach 2012). In the same cohort, 24.2% of the uncomplicated dichorionic twins were electively delivered before 38 weeks; 4.6% were delivered at 36 weeks. Among dichorionic twins, the morbidity fell from 4% among elective deliveries at 36 weeks to 1% at 38 weeks. Breathnach concluded that perinatal morbidity can be minimised by allowing uncomplicated monochorionic twin pregnancies to continue to
37 weeks and dichorionic twin pregnancies to 38 weeks (Breathnach 2012). Using this data together with the conclusions established in the NOTES Study regarding the effects of prematurity and growth discordance, the decision to deliver twins prior to 33 to 34 weeks should not be taken lightly even in the presence of significant growth discordance. A recent meta-analysis from 2016 looking at 32 studies concluded that, in the absence of complications, the optimum time for delivery of dichorionic twins appear to be around 37 weeks with 36 weeks being the optimum time for monochorionic twins. Beyond these time points, the risk of stillbirth exceeded the risk of neonatal death (Cheong-See 2016, Halling 2018). This is slightly earlier compared to Breathnach’s conclusion of ideal delivery of uncomplicated monochorionic twin pregnancies at 37 weeks and uncomplicated dichorionic twin pregnancies at 38 weeks.

BEHAVIOUR

The behavioural screening tools (Social-Emotional and Adaptive Behaviour Scales and The Child Behaviour Checklist) utilised in the NOTES Study did not demonstrate any significant differences in adverse behaviour characteristics and/or internalising and externalising behaviours between the smaller and larger twin in our growth-discordant cohort. Several smaller studies such as Monset-Couchard (2004) have demonstrated adverse behaviour outcomes in the smaller twin in growth-discordant twin pairs. This study, however, consisted mostly of growth-discordant multiples who were extremely preterm.
Adverse effects on behaviour are possibly associated with prematurity and not with growth discordance.

The NOTES Study did identify 2 children with characteristics of autism and both were subsequently diagnosed with the disorder. Although both exhibited worrying behavioural characteristics in the *Social-Emotional and Adaptive Behaviour Scales* as well as the *CBC*, suspicion first arose due to abnormal *Bayley Scales* results as well as observed behaviours during the assessment.

**CONTROL GROUP**

Assessing development is complex. Multiple factors including genetic, environmental, and socioeconomic status contribute to developmental outcomes. Twin studies are unique as inter-twin analysis allows the comparison between 2 individuals who share the same environment and socioeconomic status and are either genetically similar or identical. This enables independent factors such as growth discordance to be examined more accurately.

To add power to the NOTES Study, a control group was randomly chosen from the concordant group of twins and matched for gestation with the growth-discordant pairs. Each growth-discordant pair was matched to either one or possibly two control pairs. These control pairs were matched within one to two days of gestation to the growth-discordant pair. Because the control pairs were chosen prior to
recruitment, they were matched to the 150 growth-discordant pairs that were eligible for the NOTES Study. One hundred nineteen growth-discordant pairs were recruited. It is therefore possible that a number of the 111 control pairs are not an exact match. Despite this, the mean gestational age of the GD pairs is 35.2 and the mean GA for the control pairs is 35.4. This indicates that the growth-discordant pairs are indeed well matched for gestation with the control group.

It was expected that the growth-discordant group would perform significantly worse on the Bayley Scales compared to the control group. This did not happen. We considered the possibility that this could be explained by differences in social class, but there was no statistical difference between the social classes of the growth-discordant group and the control group (see table 7, page 85).

**ASQ-3, ITS USEFULLNESS, AND THE HIGH-RISK GROUP**

The ASQ-3 is a quick, simple, developmental screening tool. The purpose of the questionnaire is to identify those children who warrant a more detailed developmental assessment. The Bayley Scales is considered the gold standard when assessing for potential developmental delay; however, the Bayley Scales is time consuming and must be carried out by a trained professional. Only those at highest risk for developmental delay are therefore assessed. In Ireland, premature infants (usually less than 30 weeks’ gestation) receive
regular developmental follow-up from health care professionals, and this usually includes a full *Bayley Scales Assessment* around 24 months’ corrected gestational age. This type of developmental follow-up would not be feasible for every Irish child as the *Bayley Scales Assessment* must be performed by a trained health care professional and each exam takes up a significant amount of time. In 2012, 6.2% of all Irish births were preterm (delivery prior to 37 weeks’ gestation). The majority of these (5%) were born between 32 and 36 weeks’ gestation (Perinatal Statistics Report 2012). This group of children are preterm but would not qualify for a *Bayley Scales Assessment* and they therefore represent the ideal population of children who may benefit from a developmental screening tool. As the *ASQ-3* can easily be performed by parents at home, this is potentially a suitable, cost-effective, reliable screening tool for use in the general low-risk Irish population.

One of our secondary aims was to investigate the accuracy of the *ASQ-3* at correctly identifying those children with potential developmental concerns as identified through the *Bayley Scales*. With a sensitivity of 91%, a specificity of 87%, and a total agreement of 86%, we can conclude that the *ASQ-3* is indeed a safe, easy, developmental screening tool to use in low-risk Irish populations that may otherwise receive limited developmental surveillance such as late preterm twins born between 30 and 36 weeks’ gestation.

The high-risk group was identified as those concordant pairs of twins that scored poorly on the *ASQ-3*. The group was made up of 87 pairs of
twins (174 children). These children were all subsequently assessed with the Bayley Scales. Despite being identified as high risk, 133 out of 174 children still scored within the low-normal/normal range in the Bayley Scales. When compared to the growth-discordant group, however, the high-risk group scored significantly worse than the GD group in all areas of the Bayley Scales Assessment. Rates of perinatal morbidity and differences in socioeconomic status were looked at in an attempt help explain the significantly lower scores in the Bayley Scales. Only 19% of the high-risk group had perinatal morbidities, however, compared to a 37% perinatal morbidity rate in the growth-discordant group of twins and a 23% rate in the control group. In addition, no statistical differences in social class could be found between the high-risk group of twins compared to the growth-discordant or control group. This further supports the use of the ASQ-3 questionnaire in low-risk populations in Ireland that would otherwise not warrant a more detailed developmental assessment.

**LIMITATIONS OF THE NOTES STUDY**

As previously discussed, the smaller twin performed significantly worse than the larger twin in all areas of development. It is however impossible to predict if this will be of clinical significance and is thus a major limitation of the NOTES Study. The possibility that the Bayley Scales, 3rd Edition is less sensitive than the Bayley Scales, 2nd Edition at picking up developmental delays further adds to this limitation.
A further limitation of the NOTES Study is that the growth discordant group of twins were significantly younger on the day of the Bayley Scales Assessment compared to the control group of twins (34.1 versus 35.9 months). However, the Bayley Scales tasks are age dependent; the assessment compares the child to norms validated in that particular age group.

**FINAL CONCLUSIONS OF THE NOTES STUDY**

The Ages and Stages Questionnaire, 3rd Edition, can safely be used as a screening tool in low-risk populations in Ireland to identify those children who may be at risk for neurodevelopmental delays due to its high sensitivity.

The NOTES Study has demonstrated that in-utero growth discordance of 20% or more in both monochorionic and dichorionic twins puts the smaller twin at a disadvantage in terms of neurodevelopment and physical growth when compared to the larger twin at two to three years of age. The NOTES Study also demonstrated that chorionicity has an impact on development in growth-discordant twins. The NOTES Study is among the first to demonstrate that birthweight discordance confers an adverse effect on the smaller twin across all three domains of development: cognition, language, and motor (Halling 2016).

Most importantly, the NOTES Study has shown that in the absence of other complications, growth discordance alone does not justify delivery
prior to 33 weeks’ gestation as the effect of prematurity has a far more negative impact on development at this early gestation (Halling 2016).

**FURTHER RESEARCH**

A planned further neurodevelopmental assessment of this cohort in later childhood may elucidate whether adverse neurodevelopmental sequelae of twin growth discordance and prematurity progress or diminish over time.
BIBLIOGRAPHY


63. Achenbach T, Rescorla A. The Child Behaviour Checklist for ages 18 months-5. 2000


APPENDIX
Dear Parent,

Thank you for your participation in our ESPRiT national twins study during your pregnancy. The results of the study are currently being published.

Participants of the ESPRiT Study are now invited to participate in a follow-up study. This study is called The NOTES Study: Neurodevelopmental Outcome for Twins of the ESPRiT Study and is looking to investigate the development of the twins who took part in the ESPRiT Study.

Please find enclosed an information leaflet, 2 consent forms and 2 developmental questionnaires (ASQ 3rd Edition), 1 questionnaire for each twin. Please read these carefully. The information leaflet is yours to keep.

Please fill in the 2 questionnaires (one for each child) and sign the consent forms. Keep 1 consent form for your records. Please return the other consent form along with both questionnaires to me. I have included a stamped, addressed envelope for your convenience.

If you have any queries, please do not hesitate to call me at: 086 1910884.

Yours Sincerely,

Dr Cecilie Halling
Lead Researcher
Title of Study: The NOTES study: Neurodevelopmental Outcome for Twins of the ESPRiT Study

Lead Researcher: Dr Cecilie Halling

Investigators’ Name and Title: Dr Cecilie Halling, Paediatric SpR
Dr David Corcoran, Paediatric Consultant
Dr John Murphy, Paediatric Consultant
Dr Martin White, Paediatric Consultant
Dr Louise Gibson, Paediatric Consultant
Dr Orla Flanagan, Paediatric Consultant
Dr Moira Stewart, Paediatric Consultant
Dr Sinead Harty, Paediatric Consultant

Contact Details: Mobile Number: 086 19 10 884

You and your child are invited to take part in a clinical research study carried out around the country at various maternity hospitals. Before you decide whether or not you wish to take part, you should read the information provided below carefully. Take time to ask questions- do not feel rushed or obliged to make a hasty decision. You are not obliged to take part in this study and failure to participate will have no effect on your child’s future medical care. You may withdraw your child from the study at any time for whatever reason without having to justify your decision.
What is the NOTES study and why is this study being done?

As you will recall, you recently took part in the ESPRiT Study. The ESPRiT Study took place in eight major maternity centres in Ireland and it investigated difference in growth in twins. This occurs when 1 twin is smaller than the other twin. The main causes for this problem relate to poor function of the placenta (‘afterbirth’). As a participant of the ESPRiT Study, your pregnancy was closely monitored with regular ultrasound scans, looking closely at fetal growth and the placenta, investigating ways which may help to predict growth difference in twins.

The purpose of the NOTES study is to investigate the long-term developmental outcomes of the twins who took part in the ESPRiT Study.

Twins form a high-risk group of newborns because not only may there be a difference in growth between the twins, but twins also have a tendency to be born early and/or very small.

Very few studies have looked at the developmental outcomes of growth discordant twins (difference in growth) and concordant (no growth difference) twins.

Because of this we wish to assess the development of all twins who participated in the ESPRiT Study.

When will testing of my child occur?

Testing of your twins will begin when they are roughly 2 years of age. If your twins were born prematurely, they will not be tested until they reach 24 months from the time they were meant to be born.
**What does testing consist of?**

Together with this information leaflet, we will send you 2 developmental questionnaires (Ages and Stages, 3rd Edition) that we ask you to complete (1 questionnaire per twin) and return to us. This questionnaire assesses how well your child is developing for his or her age. Once again, we correct for prematurity if your child was born early. We will include a stamped envelope for your convenience. We will also include a consent form that we would like you to sign and return together with the questionnaires.

**What happens next?**

We will send you the results of the questionnaire. If your twins had significant difference in growth at birth both children will be invited to attend a detailed developmental assessment called the Bayley Scales of Infant and Toddler Development. Those twins who had no difference in growth at birth, but who may need extra help with certain areas of their development (as identified by the Ages and Stages Questionnaire), will also be invited to attend for a Bayley Scales Assessment. We will invite both twins to attend for further assessment, even if only 1 twin may need extra help.

If your twins require a Bayley Assessment, we will contact you via telephone. The Bayley Assessment will take approximately 30 – 40 minutes per child to complete. If you are from Dublin, the assessment will take place either in the Rotunda Hospital or the National Maternity Hospital. The assessment will take place locally if you are from outside of Dublin.

**What happens if the Bayley assessment suggests that my child needs extra help in one or more areas of development?**

We will refer you on to appropriate services that will help your child with his/her development.
What happens if my child has already had a Bayley Scales Assessment?
If your child was assessed within the past 4 months, we will use this result and we will not put your child through another assessment.

How will taking part in this study benefit my child?
The NOTES study is the largest neurodevelopmental follow-up study of twins ever to be conducted in the Irish population. This study will therefore give us an insight into possible developmental problems some twins might encounter as young children. At the end of this study, we will therefore be in a better position to deliver the appropriate care and services to these children.

If your child is identified as needing extra help in an area of development, we will make sure that your child is referred to the appropriate services so your child will receive the help he/she needs.

If you require further information:
If you have any further questions about this study, or if you wish to have your child withdrawn from the study, you may do so without justifying your decision.

For additional information please contact:

Dr Cecilie Halling at 086 1910884
CONSENT FORM

For Participation in the: NOTES study: Neurodevelopmental Outcome for Twins of the ESPRiT Study

Lead Researcher: Dr Cecilie Halling

Contact Details: 086 19 10 884

Please circle appropriate answer

1. Have you read the information leaflet about this study? Yes / No

2. Has a researcher spoken to you about this study and answered any questions you had? Yes / No

3. Do you allow your children to take part in this study? Yes / No

4. Do you agree to have your children’s medical records accessible to the research team? Yes / No

5. Do you consent to having your child undergo a detailed developmental assessment (Bayley Scales Assessment) if deemed beneficial by the research team? Yes / No

Your child’s medical records will only be accessible to the Research team. Information will be stored on a database. This database is confidential and anonymous as each child will be assigned a study number and no names will be used. This database will be kept for 2 years after the study is complete at which time it will be destroyed.
<table>
<thead>
<tr>
<th>Name of Parent and Children</th>
<th>Date</th>
<th>Signature of Parent</th>
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<tr>
<th>Researcher Name</th>
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Neuro-developmental outcome of a large cohort of growth discordant twins

Cecille Halling1,7 • Fergal D. Malone1 • Fionnuala M. Breathnach1 • Moira C. Stewart2 • Fionnuala M. McAuliffe3 • John J. Morrison4,5 • Patrick Dicker6 • Fiona Manning6.
John David Corcoran1 on behalf of Perinatal Ireland Research Consortium

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Abstract Our aims were to study the effect of birthweight growth discordance (≥20 %) on neuro-developmental outcome of monochorionic and dichorionic twins and to compare the relative effects of foetal growth discordance and prematurity on cognitive outcome. We performed a cross-sectional multicentre prospective follow-up study from a cohort of 948 twin pregnancies. One hundred nineteen birthweight-discordant twin pairs were examined (24 monochorionic pairs) and were matched for gestational age at delivery with 111 concordant control pairs. Participants were assessed with the Bayley Scales between 24 and 42 months of age. Analysis was by paired t test for intra-twin pair differences and by multiple linear regression. Compared to the larger twin of a discordant pair, the smaller twin performed significantly worse in cognition (mean composite cognitive score difference−1.7, 95 % confidence interval (CI)=0.3−3.1, p=0.01) and also in language and motor skills. Prematurity prior to 33 weeks’ gestation, however, had a far greater impact on cognitive outcomes (mean cognitive composite score difference−5.8, 95 % CI=−1.2−10.5, p=0.008).

Conclusion: Birthweight growth discordance of ≥20 % confers an independent adverse effect on long-term neuro-development of the smaller twin. However, prior to 33 weeks’ gestation, gestational age at birth adversely affects cognitive development to a greater extent than foetal growth discordance.

Communicated by Peter de Winter

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Introduction

Twin pregnancy is associated with a threefold to seventofold increase in perinatal morbidity and mortality compared to singleton pregnancies [13] and with a fourfold increase in neurologic morbidity [11, 16]. Growth discordance, whereby foetal growth is significantly mismatched within a twin pair, is a complication of both monochorionic (MC) and dichorionic (DC) twin pregnancies. In addition to an increased risk of perinatal morbidity (RDS, IVH, NEC) [6, 26], severe growth discordance may also result in adverse long-term neuro-developmental consequences [12, 14, 18].

Among healthy twins, growth discordance may be due to the inability of the uterine environment to meet the demands of multiple foetuses, leading to intrauterine growth restriction [5]. Twin-twin transfusion syndrome (TTTS) is a complication unique to MC twins, occurring in 10–15% of these pregnancies [22], due to arteriovenous anastomoses in the shared placenta leading to imbalanced blood flow. TTTS may result in foetal loss of one or both twins and increase the risk of perinatal morbidity as well as increasing the risk of developing cerebral palsy [22].

Methods

The Neuro-Developmental Outcome for Twins of the ESPRIT Study (NOTES Study) was a cross-sectional multicentre prospective follow-up study investigating neuro-developmental outcome in twins. Participants were recruited from the ESPRIT twin study (Evaluation of Sonographic Predictors of Restricted Growth in Twins) [6, 7] and included both MC and DC twins. Chorionicity was established at booking ultrasound prior to 14 weeks’ gestation and was confirmed by histopathological examination of the placenta after delivery. Monoamniotic twins were excluded from both studies. Our study did not include single-twin survivors because of the high risk of neurological impairment in single-twin survivors of MC pregnancies. From a cohort of 1921 children (948 pairs of twins and 25 single survivors of twin pregnancies), 159 pairs of twins with at least 20% birthweight discordance (as defined at the start of the ESPRIT twin study) were identified. A further 120 concordant (less than 20% birthweight discordance) control pairs were randomly selected and matched with the discordant cases for gestational age at birth. Figure 1 illustrates the numbers of twin pair participants in both the ESPRIT
twin study and the NOTES study. Assuming 80% statistical power, a sample size of 120 twin pairs per study group would detect a minimum intra-twin difference of 2 points on a composite score from the Bayley Scales of Infant and Toddler Development, 3rd edition [3], assuming a standard deviation of 8 points within twin pairs and a nominal 5% level of significance.

All eight academic tertiary referral perinatal centres in Ireland were involved in the NOTES Study. Ethics approval was obtained at each institution. Recruitment ran from July 2010 through to June 2012. Participants were between 24 and 42 months of age on the day of assessment. Age was adjusted for preterm birth if delivery occurred before 37 weeks’ gestation.

Participants were initially contacted via telephone by the lead investigator or by a sonographer involved in the ESPRiT twin study. An information leaflet and consent form was posted to each participant along with a stamped addressed envelope to facilitate return of signed consent forms to the lead researcher. Once the 120 control pairs had been randomly selected, the growth discordant pairs and the control pairs were invited to attend for assessment. Each twin pair was assessed with the Bayley Scales of Infant and Toddler Development, 3rd edition [3]. A total of four examiners across Ireland carried out all the assessments. These examiners were trained professionals (psychologists or research nurses) or the lead researcher. Because the Bayley Scales were performed by more than one individual, inter-rater reliability was assessed using a random effects model. The examiners were unaware of the discordance status of the twins, their birth history and gestational age at birth. The Bayley Scales Social Emotional and Adaptive Behaviour Questionnaire and the Child Behaviour Checklist [1] were completed by the parents.

Composite and scaled scores from the Bayley Scales across five domains (cognitive, language, motor, social-emotional and adaptive behaviour) and the Child Behaviour Checklist scores were compared between growth discordant infants (intra-twin analysis) and also between growth discordant pairs and controls.

We also compared socio-economic status between the growth discordant and the control group of twins. Social class was defined as the maximum social class of the household (i.e. maximum social class of mother and father). Social class categories were defined according to the Soc90 classification used in the UK and Ireland [24]. The categories were further combined as either professional/skilled (managerial, professional or technical) or non-professional/unskilled (manual, semi-skilled or unskilled).
The effects of chorionicity, gender, prematurity and birthweight (small for gestational age (SGA <5th centile as defined by the ESPRIT twin study) were also analyzed. The RCPCH UK-WHO growth charts were used to plot weight. To account for correlations within twin pairs, a paired t test was used for intra-twin comparisons while a linear mixed effects model (with twin pair as a random effect to account for intra-twin correlations) was used for multiple linear regression analysis of developmental outcomes. Growth discordant status (either control twin pairs or growth discordant pairs) was included as a factor in the multiple linear regression while chorionicity and prematurity were included as covariates. For comparisons of maternal and twin pair demographics, unpaired t tests were used. All statistical analyses and screening of data were performed using the SAS Software® Version 9.1.

Graphical descriptions of the data were performed using the R Software version 2.15.

**Results**

Nine hundred and forty-eight pairs of twins from the ESPRIT twin study were alive at 2 years of age. Of these, 159 were growth-discordant at birth. Nine pairs were excluded due to the following: chromosomal abnormalities (1), autism (3), intraternal demise of one twin (1) and postnatal death (4). Thirty-two of the remaining 150 pairs were MC (see Fig. 1). Owing to the prevalence of single- or dual-fetal demise in the setting of TTTS, only three MC growth discordant twin pairs in the NOTES Study met criteria for a diagnosis of TTTS.

**Table 1** Demographics of growth-discordant twin pairs and control pairs

<table>
<thead>
<tr>
<th></th>
<th>Growth-discordant pairs (N=119)</th>
<th>Control pairs (N=111)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age (weeks)</strong></td>
<td>35.2 (±2.7)</td>
<td>35.4 (±2.6)</td>
<td>0.55</td>
</tr>
<tr>
<td>GA &lt;30 weeks</td>
<td>7 (6 %)</td>
<td>6 (6 %)</td>
<td></td>
</tr>
<tr>
<td>30&lt;GA&lt;32 weeks</td>
<td>87 (7 %)</td>
<td>5 (5 %)</td>
<td></td>
</tr>
<tr>
<td>32&lt;GA&lt;34 weeks</td>
<td>13 (11 %)</td>
<td>15 (14 %)</td>
<td></td>
</tr>
<tr>
<td>34&lt;GA&lt;36 weeks</td>
<td>33 (28 %)</td>
<td>27 (24 %)</td>
<td></td>
</tr>
<tr>
<td>36&lt;GA&lt;37 weeks</td>
<td>22 (18 %)</td>
<td>22 (20 %)</td>
<td></td>
</tr>
<tr>
<td>GA ≥37 weeks</td>
<td>36 (30 %)</td>
<td>36 (32 %)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean birthweight (g) (SD)</strong></td>
<td>322 (±700)</td>
<td>2370 (±335)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>MCDA pairs</strong></td>
<td>33.6 (±3.1)</td>
<td>35.4 (±2.8)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Mean age</strong></td>
<td>35.6 (±1.8)</td>
<td>35.4 (±2.8)</td>
<td></td>
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<tr>
<td><strong>AGA pairs</strong></td>
<td>73 (61 %)</td>
<td>106 (95 %)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>SGA/AGA pairs</strong></td>
<td>44 (37 %)</td>
<td>4 (4 %)</td>
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<td><strong>SGA pairs</strong></td>
<td>2 (2 %)</td>
<td>1 (1 %)</td>
<td></td>
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<tr>
<td><strong>Elective Caesarean delivery</strong></td>
<td>70 (59 %)</td>
<td>44 (40 %)</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Emergency pre labour Caesarean delivery</strong></td>
<td>21 (18 %)</td>
<td>11 (10 %)</td>
<td></td>
</tr>
<tr>
<td><strong>Assisted conception</strong></td>
<td>37 (33.3 %)</td>
<td>28 (27.1 %)</td>
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<tr>
<td><strong>Maternal age</strong></td>
<td>33.5 (±5.3)</td>
<td>33.0 (±5.0)</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Maternal BMI</strong></td>
<td>25.7 (±4.5)</td>
<td>26.1 (±5.0)</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Caucasian ethnicity</strong></td>
<td>112 (94 %)</td>
<td>105 (95 %)</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Social class (professional)</strong></td>
<td>55 (54 %)</td>
<td>69 (63 %)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Neonatal morbidity</strong></td>
<td>17 (14 %)</td>
<td>10 (9 %)</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Age on day of Bayley assessment in months (range)</strong></td>
<td>34.1±4.3 (24–42)</td>
<td>35.9±3.9 (24–42)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Weight discordance at assessment (%)</strong></td>
<td>10.1±5.8 (4–24)</td>
<td>7.1±5.8 (4–24)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Weight discordance at assessment &gt;20 %</strong></td>
<td>13 (11 %)</td>
<td>4 (4 %)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

**AGA** appropriately grown for gestational age, **SGA** small for gestational age

a Percentages for completed weeks of gestation are cumulative

b BW both twins above 5th centile
c One twin BW above 5th centile; other twin BW below 5th centile
d Includes IVF and ICSI
e Social class was unobtainable for nine (8 %) of the GD group and ten (9 %) of control group. “Professional” was defined as managerial, professional or technical (skilled)
f Composite outcome of IVH, HIE, radiological evidence of PVL or NEC
We recruited 119 out of the 150 growth discordant pairs (79% follow-up rate), of which 24 were MC. One hundred and eleven control pairs were consented to recruitment (see Fig. 1). A total of 460 Bayley Scales Assessments were carried out. Inability to complete the entire exam on both twins on the same day occurred on two occasions. In both cases, the twins were assessed successfully 6 months later.

Infant demographics for each group are illustrated in Table 1. An inter-rater reliability was performed, and we found no evidence of systematic differences in ratings for any item of the Bayley Scales—a maximum of only 1% of the total variability was due to examiner differences.

Figure 2 illustrates the mean differences in Bayley Scales scores between larger and smaller twins (larger minus smaller) in the growth discordant and concordant (control) groups of twins which are not directly compared. Among growth discordant infants, the smaller twin had lower scores than the larger twin in cognition (mean difference in composite scores = −1.7, 95% confidence interval (CI) = −3.1 to −0.3, p value = 0.02), composite language (mean difference = −1.8, 95% CI = −3.3 to −0.3, p value = 0.02) and composite motor category (mean difference = −2.2, 95% CI = −4.0 to −0.4, p = 0.02). In addition, the smaller twin had lower scaled scores than the larger twin in the areas of receptive communication (mean difference = −0.3, 95% CI = −0.6 to −0.1, p = 0.04) and fine motor category (mean difference = −0.4, 95% CI = −0.8 to −0.01, p = 0.02). There was no difference in the scaled expressive language category (mean difference = −0.2, 95% CI = −0.5 to 0.09, p value = 0.15) or the scaled gross motor category (mean difference = −0.3, 95% CI = −0.7 to 0.1, p value = 0.19) (Fig. 2).

There was no difference in social-emotional and adaptive behaviour or in inactivating or externalizing behaviours, as identified through The Child Behaviour Checklist, between

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**Fig. 2** Boxplots showing Bayley Scales score results for twins in the NOTES Study. Intra-twin mean difference in Bayley Scales scores for discordant and concordant twin pairs (p values, paired t-test at different gestational age cut-offs. Boxplots represent 25th and 75th centiles (box), 50th centile (box internal line) and 10th and 90th centiles (extent of whiskers).
Table 2  Bayley Scales Assessment results for growth-discordant monochorionic twins vs growth-discordant dichorionic twins

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Dichorionic mean (N=95)</th>
<th>Monochorionic mean (N=24)</th>
<th>Difference* (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive composite score</td>
<td>102.0</td>
<td>100.7</td>
<td>1.3 (-1.7, 4.3)</td>
<td>0.41</td>
</tr>
<tr>
<td>Language composite score</td>
<td>106.7</td>
<td>103.0</td>
<td>3.8 (0.4, 7.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>Expressive language scaled score</td>
<td>11.0</td>
<td>10.2</td>
<td>0.8 (0.2, 1.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Receptive language scaled score</td>
<td>11.3</td>
<td>10.8</td>
<td>0.5 (-0.1, 1.1)</td>
<td>0.09</td>
</tr>
<tr>
<td>Motor composite score</td>
<td>106.6</td>
<td>101.4</td>
<td>5.3 (2.0, 8.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fine motor scaled score</td>
<td>11.0</td>
<td>10.4</td>
<td>0.6 (-0.1, 1.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>Gross motor scaled score</td>
<td>11.2</td>
<td>10.0</td>
<td>1.1 (0.5, 1.8)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Adjusted for gestational age at delivery and incorporating intra-twin correlations (mixed-effect model)

the smaller and larger twins. There was also no difference in development or behavioural problems between same-sex growth discordant pairs and different-sex growth discordant pairs.

Growth-discordant MC twins had lower scores than DC growth discordant twins in composite language, scaled expressive language, composite motor and scaled gross motor categories (p<0.05) (Table 2). Small sample size precluded analysis of significant differences between the smaller and larger twins in growth-discordant MC pairs (N=24).

There were no differences in any parameter of development between the control group and the smaller twin in growth discordant pairs nor was there any difference between average scores of the control group and average scores of the growth discordant groups after adjusting for gestational age at delivery, social class and intra-twin correlations.

Finally, we compared the impact of intra-uterine growth discordance and prematurity by a multiple regression analysis of composite cognitive score of the growth discordant group (N=119 pairs) showing the effect of growth discrepancy in twin pairs below gestational age cut-offs and showing the effect of prematurity following adjustment for growth discrepancy, incorporating intra-twin correlations using a mixed effects model (Fig. 3). This shows the effect of growth discordance on cognitive outcome between the smaller and larger twins to be small but relatively constant as gestation increases (dashed lines), while the effect of gestation (solid lines) below 33 weeks has a far greater negative impact on cognitive outcomes (mean cognitive composite score difference—5.8, 95% CI—1.2 to 10.5, p=0.008).

Discussion
Our study is amongst the first to demonstrate that the smaller twin in a growth discordant pair will have lower neurodevelopmental scores across all three domains of development: cognition, language and motor. More importantly, our study is amongst the first to demonstrate that the clinical
impact of growth discordance on cognition is small in comparison to the effects of preterm birth.

This study represents one of the largest studies undertaken to investigate neurodevelopmental outcome of growth discordant twins. It is uniquely placed in allowing examination of growth discordance as an independent risk factor for adverse developmental outcomes as well as quantifying this risk by comparing growth discordance to other factors such as preterm birth. Studies of long-term outcomes in growth-discordant twins have not shown consistent relationships between birthweight discordance and subsequent neurodevelopment [9, 12, 14, 18, 21]. Goyen et al. [12] assessed 21 pairs of growth-discordant very low birthweight (VLBW) preterm twins at 3 years of age and found that the smaller twin scored significantly lower in the locomotor subscale compared to the larger co-twin. Monest-Couchard [18] studied 36 pairs of twins, where the smaller twin was small for gestational age (SGA) and <1 kg at birth and the larger twin was appropriately grown for gestational age (AGA) and reported no difference in locomotor scores but found that the lighter twin remained smaller at 3 years of age and also had more visual, behavioural and speech problems. Our study demonstrates a negative impact of growth discordance on the smaller twin across all three domains of development: cognition, language and motor.

Chorionicity represents a putative confounder in the prevalence of neurologic morbidity among twin survivors. MC twins have higher rates of foetal loss, extreme prematurity and neonatal morbidity [13, 22]. This risk is mainly attributed to TTTS, but even in its absence, MC twins are at increased risk of adverse neurological outcome. Hack et al. [14] demonstrated a significantly higher incidence of mildly delayed development of hearing and language in uncomplicated MC twins at 2 years of age compared to DC twins. In our study, MC twins had lower scores than DC pairs, following adjustment for gestation, in the areas of language and fine and gross motor function.

We did not observe any significant differences in adverse behaviour characteristics between the smaller and larger twins.

We were unable to demonstrate higher rates of mild, moderate or severe developmental delays in the smaller twin in our growth-discordant pairs, using the EPICure criteria for developmental delay [17]. One twin (the smaller twin) in the growth-discordant group suffered from bilateral hearing loss requiring hearing aids. However, the Bayley Scales, 3rd edition, possibly overestimate cognitive outcomes [2, 25] and some subjects in our study may have more significant cognitive delays at school age. In a study confined to 71 monozygotic twin pairs studied between ages 7 and 18, Edmondson et al. [10] demonstrated that the smaller twin had a progressively lower verbal intelligence quotient as the degree of growth discrepancy increased. It is likely that the differences observed in cognition, language and motor scores in our study will manifest to some degree as small but significant differences in intelligence quotients in later life.

A further limitation is that the growth-discordant group of twins was significantly younger on the day of assessment compared to the control group of twins (34.1 versus 35.9 months, see Table 1). However, the Bayley Scales tasks are age dependent; the assessment compares the toddler to norms validated in that particular age group.

The strengths of this study lie in a high follow-up rate of a large growth-discordant cohort (79%) which allows us to accurately interrogate growth discordance and prematurity as independent predictors of neurodevelopmental outcome in twin pregnancies. From singleton studies, we already know that preterm and SGA infants are at a neurodevelopmental disadvantage, even amongst the late preterm group of twins (32-36 weeks) [8, 19, 20, 23]. Our study, however, shows that growth discordance has a negative effect on neurodevelopmental outcome of the smaller twin, whether preterm or near term, which also affects AGA infants and is independent of perinatal environmental factors. It is likely that this is due to the adverse effect of intrauterine growth restriction on the smaller twin [9, 10, 12, 14, 21, 23].

Most importantly, we were able to compare the effect of growth discordance on cognitive outcome to the effects of prematurity. Although growth discordance has a small negative effect on cognitive outcome, this effect seems constant regardless of gestation, while the impact of prematurity at <33 weeks' gestation is far greater than the effect of discordant foetal growth. This suggests that growth discordance in isolation is an insufficient indication to deliver twins prematurely.

Our study has established the relative risk of growth discordance in relation to the degree of prematurity on cognitive outcome. A planned further neurodevelopmental assessment of this cohort in later childhood may elucidate whether adverse neurodevelopmental sequelae of twin growth discordance and prematurity progress or diminish over time.

Birthweight discordance of 20% or more in both MC and DC twin pairs places the smaller twin at a neurodevelopmental disadvantage when compared to the larger twin at 2 to 3.5 years of age. Chorionicity has an impact on development. Significant growth discordance alone does not justify delivery prior to 33 weeks' gestation, as the effect of prematurity has a far more negative impact on development at this early gestation.

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Author’s contributions Cecilia Halling (first author): Dr Halling conceptualized and designed the study, coordinated all data collection, drafted the initial manuscript and wrote the final manuscript with contributions from other authors.

John David Corcoran (senior author): Dr Corcoran conceptualized and helped design the study, supervised the study, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

Fergal Malone: Dr Malone conceptualized and helped design the study, reviewed the initial manuscript and approved the final manuscript as submitted.

Fionnuala Breathnach: Dr Breathnach conceptualized and helped design the study, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

Moira Stewart: Dr Stewart conceptualized and helped design the study, reviewed the initial manuscript and approved the final manuscript as submitted.

Fionnuala McAmull: Dr McAmull conceptualized and helped design the study, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

John J Morrison: Dr Morrison conceptualized and helped design the study, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

Fiona Manning: Ms Manning conceptualized and helped design the study, helped with the grant application process, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

Patrick Dicker: Mr Dicker conceptualized and helped design the study, designed the data collection instruments, carried out all statistical analysis for the study and approved the final manuscript as submitted.

Compliance with ethical standards

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Conflict of interest Author A declares that she has no conflict of interest. Author B declares that she has no conflict of interest. Author C declares that he has no conflict of interest. Author D declares that she has no conflict of interest. Author E declares that she has no conflict of interest. Author F declares that he has no conflict of interest. Author G declares that he has no conflict of interest. Author H declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Little and large: the effects of twin growth discordance

Cecilia Halling,1,2 John David Corcoran1,3

Twin and higher order multiples have a natural frequency of <2% of live born infants. As in vitro fertilisation (IVF) techniques have become more widespread, the frequency of multiple births has more than doubled, with the largest increase being in dichorionic pregnancies. Women who have twins tend to be older and are at higher risk of associated health problems and pre-eclampsia than the general population. There is a higher rate of late fetal loss in multiple pregnancies, particularly in monochorionic pregnancies, and fetal growth is also reduced in multiple pregnancies in the third trimester. These factors lead to a high rate of preterm birth in multiple pregnancies, even when one considers that 38 weeks is generally considered as term for twin pregnancies.

The high rate of preterm delivery in multiples is reflected in the workload of neonatal units, with about 30% of infants <1.5kg being twins or higher order multiples.1 The medical costs associated with twins in the first 5 years of life are three times that of singleton infants, with most of these costs being incurred due to neonatal admissions because of prematurity.2

Swamy et al report on the developmental outcome at school age of 51 pairs of monochorionic twins with growth discrepancy ranging from 20% to 56%.3 The authors are to be congratulated for a high follow-up rate of 77%. The cohort had a mean gestation of 34.7 weeks with a mean difference in birth weight of 64g. The lighter twin had a General Conceptual Ability (GCA) score that was three points lower (small twin 105.4 vs large twin 108.4, 95%CI – 0.9 to – 5.0). Mathematics and memory scores showed the largest differences, and there was a significant positive association of birth weight differences and GCA scores.

Their data set contains a number of pregnancies complicated by twin-twin transfusion syndrome.4 These findings are consistent with the previously published studies in the area. Edmonds et al studied 71 monozygotic twin pairs between the ages of 7 and 18 and found a relationship between the severity of the growth discrepancy and lower verbal IQ in the smaller twin.5 Several other studies have reported similar findings with developmental disadvantage reported in the smaller twin, though not all have confirmed choriocity. In the largest published study of growth-discordant twins, Halling et al, using a similar definition of growth discrepancy to Swamy et al, assessed 119 twin pairs at 2-3 years of age (20% were monochorionic) and documented small but significant differences between the larger and smaller twin on Bayley scale assessment in the areas of cognition and in the composite language and motor categories.6 The statistical analysis of twins is fraught with difficulties, but despite this, the weight of evidence suggests that growth-restricted twins, whether mono or dichorionic, are at a slight developmental disadvantage to their larger counterparts which persists into adolescence.

Monochorionic twin pregnancies, however, are associated with increased risk compared with dichorionic pregnancies. Because of shared placentaion, the fate of both twins is inextricably linked, which alters perinatal management at the edges of viability. They are more likely to be delivered prematurely and thus are more vulnerable to neonatal complications. They have a higher rate of congenital abnormality. In the monochorionic cohort reported in this edition by Swamy, 2% of the infants had cerebral palsy.7 Hack et al studied developmental outcomes and cerebral palsy rates in a cohort matched for gestation, gender and weight (n=366). Four out of 182 monochorionic infants had cerebral palsy (2.2%) attributed to complications of twin–twin transfusion syndrome, intrauterine demise of a co-twin or complications of prematurity compared with one dichorionic twin (0.5%), a non-significant difference. The majority of 2-year-old twins who underwent developmental assessments (n=282) had normal developmental status. Nor were there significant differences in developmental outcome between monochorionic and dichorionic twins. Birth weight discordancy did not influence long-term outcome, though the smaller twin had slightly lower developmental scores than its larger co-twin.8 Halling et al have reported that growth-discordant monochorionic twins have lower composite language and motor scores than growth discordant dichorionic twins, after adjustment for gestational age and intra-twin correlations, though there was no significant difference in cognitive scores (n=119).9

The negative effect of growth discordancy on monochorionic twins is well known to obstetricians. Brana et al studied twins born in the USA between 1995 and 1997.10 Among the 8% of the twin sets with 25% birth weight discordance or more, there was a significant increase in neonatal mortality which persisted after adjustment for fetal growth and prematurity. Four per cent of the population had growth discordance of 30% or more; in this group, the mortality in the smaller twins was 11 times higher than the observed mortality in the smaller twin when growth discordance was <15%.

Close monitoring of twin pregnancies has improved these outcomes, but chorionicity remains a predictor of complications. In the prospective cohort study (n=1028, 20% monochorionic) reported by Breathnach et al, mothers were offered regular fetal monitoring. Nevertheless, the overall perinatal mortality of 3% in normally formed monochorionic twins >500g was seven times higher than that in dichorionic twins. Part of this excess mortality is explained by a preterm delivery rate of 20% of monochorionic twins at or before 32 weeks of gestation (compared with 7% of dichorionic twins) due to complications such as severe growth discrepancy or twin-twin transfusion syndrome.

In the absence of complications, however, intratropical delivery should be avoided. A recently published meta-analysis of 32 studies, including over 35 000 pregnancies, assessed the risks of stillbirth and neonatal complications in uncomplicated monochorionic and dichorionic twins. Monoamnionicity and twin–twin transfusion syndrome were exclusion criteria. In this population, the optimum time for delivery of dichorionic twins appears to be 37 weeks, with 36 weeks being the optimum time for monochorionic twins. Beyond these time points, the risk of stillbirth exceeded the risk of neonatal death. These conclusions,
however, are tempered by the fact that some stillbirths in the cohort were likely to have been prevented by preterm delivery. Those tempted to deliver twins prematurely because of uncomplicated growth discordance should be aware of the findings of the study of Halling et al. The authors compared the impact of in utero growth discordance (20% or more) and prematurity by a multiple regression analysis, incorporating intra-twin correlations using a mixed effects model, analysing the effect of growth discrepancy in twin pairs below gestational age cut-offs and showing the effect of prematurity following adjustment for growth discrepancy. When compared with the larger twin of a discordant pair, the smaller twin performed significantly worse in cognition (mean composite cognitive score difference $-1.7 (95\% CI 0.3$ to $3.1, p=0.011$). Prematurity prior to 33 weeks’ gestation, however, had a far greater impact on cognitive outcomes (mean cognitive composite score difference $-5.8, 95\% CI 1.2$ to $10.5, p=0.008$), suggesting the adverse effects of being born before 33 weeks gestation far outweighs the negative effect on development associated with intrauterine growth restriction.

Fetomaternal medicine specialists face a dilemma in the management of twin pregnancies and must balance the short-term and long-term risks of prematurity against the risk of intrapartum death of one or both twins, depending on choriocity. The evidence suggests that uncomplicated twin pregnancies should be let proceed to 36 weeks if monochorionic and 37 weeks if dichorionic, while the presence of growth discrepancy of over 25% may be an indication for delivery, especially if the smaller twin is compromised.

The study of Swamy et al. is a valuable confirmation that the negative effect of intrapartum growth restriction on the development of genetically identical monochorionic twins persists up to school age. However, this risk must be balanced against the developmental consequences of prematurity. Close monitoring of twin growth and objective use of ultrasonic markers of fetal well-being is required to ensure optimum timing of delivery and to minimise iatrogenic preterm twin births.

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**REFERENCES**


### COMMUNICATION

1. Without your showing him, does your child point to the correct picture when you say, “Show me the kitty,” or ask, “Where is the dog?” (She needs to identify only one picture correctly.)

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
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<tbody>
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</table>

2. Does your child initiate a two-word sentence? For example, when you say a two-word phrase, such as “Mama eat,” “Daddy play,” “Go home,” or “What’s this?” does your child say both words back to you? (Mark “yes” even if her words are difficult to understand.)

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
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</table>

3. Without your giving him clues by pointing or using gestures, can your child carry out at least three of these kinds of directions?

   - a. “Put the toy on the table.”
   - b. “Close the door.”
   - c. “Bring me a towel.”
   - d. “Find your coat.”
   - e. “Take my hand.”
   - f. “Get your book.”

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
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4. If you point to a picture of a ball (kitty, cup, hat, etc.) and ask your child, “What is this?” does your child correctly name at least one picture?

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
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5. Does your child say two or three words that represent different ideas together, such as “See dog,” “Mommy come home,” or “Kitty gone”? (Don’t count word combinations that express one idea, such as “bye-bye,” “all gone,” “all right,” and “What’s that?”) Please give an example of your child’s word combinations:

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
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</table>
COMMUNICATION

6. Does your child correctly use at least two words like “me,” “I,” “mine,” and “you”? ________________

GROSS MOTOR

1. Does your child walk down stairs if you hold onto one of her hands? She may also hold onto the railing or wall. (You can look for this at a store, on a playground, or at home.) ________________

2. When you show your child how to kick a large ball, does he try to kick the ball by moving his leg forward or by walking into it? If your child already kicks a ball, mark “yes” for this item. ________________

3. Does your child walk either up or down at least two steps by herself? She may hold onto the railing or wall. ________________

4. Does your child run fairly well, stopping herself without bumping into things or falling? ________________

5. Does your child jump with both feet leaving the floor at the same time? ________________

6. Without holding onto anything for support, does your child kick a ball by swinging his leg forward? ________________

GROSS MOTOR TOTAL

*If Gross Motor Item 6 is marked “yes” or “sometimes,” mark Gross Motor Item 2 “yes.”
24 Month Questionnaire

FINE MOTOR

1. Does your child get a spoon into his mouth right side up so that the food usually doesn't spill?
   - Yes:  
   - Sometimes:  
   - Not Yet:  

2. Does your child turn the pages of a book by herself? (She may turn more than one page at a time.)
   - Yes:  
   - Sometimes:  
   - Not Yet:  

3. Does your child use a turning motion with his hand while trying to turn doorknobs, wind up toys, twist tops, or screw lids on and off jars?
   - Yes:  
   - Sometimes:  
   - Not Yet:  

4. Does your child flip switches off and on?
   - Yes:  
   - Sometimes:  
   - Not Yet:  

5. Does your child stack seven small blocks or toys on top of each other by herself? (You could also use spoons of thread, small boxes, or toys that are about 1 inch in size.)
   - Yes:  
   - Sometimes:  
   - Not Yet:  

6. Can your child string small items such as beads, macaroni, or pasta "wagon wheels" onto a string or shoelace?
   - Yes:  
   - Sometimes:  
   - Not Yet:  

FINE MOTOR TOTAL

PROBLEM SOLVING

1. After watching you draw a line from the top of the paper to the bottom with a crayon (or pencil or pen), does your child copy you by drawing a single line on the paper in any direction? (Mark "yes" if your child scribbles back and forth.)
   - Yes:  
   - Sometimes:  
   - Not Yet:  

2. After a crumb or Cheerio is dropped into a small, clear bottle, does your child turn the bottle upside down to dump out the crumb or Cheerio? (Do not show him how.) (You can use a soda-pop bottle or baby bottle.)
   - Yes:  
   - Sometimes:  
   - Not Yet:  

3. Does your child pretend objects are something else? For example, does your child hold a cup to her ear, pretending it is a telephone? Does she put a box on her head, pretending it is a hat? Does she use a block or small toy to stir food?
   - Yes:  
   - Sometimes:  
   - Not Yet:  

4. Does your child put things away where they belong? For example, does he know his toys belong on the toy shelf, his blanket goes on his bed, and dishes go in the kitchen?
   - Yes:  
   - Sometimes:  
   - Not Yet:  

5. If your child wants something she cannot reach, does she find a chair or box to stand on to reach it? (For example, to get a toy on a counter or to "help" you in the kitchen?)
   - Yes:  
   - Sometimes:  
   - Not Yet:  

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PROBLEM SOLVING (continued)

6. While your child watches, line up four objects like blocks or cars in a row. Does your child copy or imitate you and line up four objects in a row? (You can also use spools of thread, small boxes, or other toys.)

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<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PROBLEM SOLVING TOTAL

PERSONAL-SOCIAL

1. Does your child drink from a cup or glass, putting it down again with little spilling?

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Does your child copy the activities you do, such as wipe up a spill, sweep, shave, or comb hair?

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Does your child eat with a fork?

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. When playing with either a stuffed animal or a doll, does your child pretend to rock it, feed it, change its diapers, put it to bed, and so forth?

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Does your child push a little wagon, stroller, or other toy on wheels, steering it around objects and backing out of corners if he cannot turn?

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Does your child call herself “I” or “me” more often than her own name? For example, “I do it,” more often than “Juana do it.”

<table>
<thead>
<tr>
<th>YES</th>
<th>SOMETIMES</th>
<th>NOT YET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PERSONAL-SOCIAL TOTAL

OVERALL

Parents and providers may use the space below for additional comments.

1. Do you think your child hears well? If no, explain:

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Do you think your child talks like other toddlers her age? If no, explain:

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
OVERALL (continued)

3. Can you understand most of what your child says? If no, explain:
   □ YES  □ NO

4. Do you think your child walks, runs, and climbs like other toddlers his age?
   If no, explain:

5. Does either parent have a family history of childhood deafness or hearing
   impairment? If yes, explain:

6. Do you have any concerns about your child’s vision? If yes, explain:
   □ YES  □ NO

7. Has your child had any medical problems in the last several months? If yes, explain:
   □ YES  □ NO
8. Do you have any concerns about your child’s behavior? If yes, explain:
   ○ YES  ○ NO

9. Does anything about your child worry you? If yes, explain:
   ○ YES  ○ NO
# Child Behavior Checklist for Ages 1½ - 5

Please fill out this form to reflect your view of the child's behavior even if other people might not agree. Feel free to write additional comments beside each item and in the space provided on page 2. Be sure to answer all items.

Below is a list of items that describe children. For each item that describes the child now or within the past 2 months, please circle the 2 if the item is very true or often true of the child. Circle the 1 if the item is somewhat or sometimes true of the child. If the item is not true of the child, circle the 0. Please answer all items as well as you can, even if some do not seem to apply to the child.

## Checklist Items

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Achy or pains (without medical cause; do not include stomach or headaches)</td>
</tr>
<tr>
<td>2</td>
<td>Acts too young for age</td>
</tr>
<tr>
<td>3</td>
<td>Afraid to try new things</td>
</tr>
<tr>
<td>4</td>
<td>Avoids looking others in the eye</td>
</tr>
<tr>
<td>5</td>
<td>Can't concentrate, can't pay attention for long</td>
</tr>
<tr>
<td>6</td>
<td>Can't sit still, restless, or hyperactive</td>
</tr>
<tr>
<td>7</td>
<td>Can't stand having things out of place</td>
</tr>
<tr>
<td>8</td>
<td>Can't sit waiting; wants everything now</td>
</tr>
<tr>
<td>9</td>
<td>Chews on things that aren't edible</td>
</tr>
<tr>
<td>10</td>
<td>Clings to adults or too dependent</td>
</tr>
<tr>
<td>11</td>
<td>Constantly seeks help</td>
</tr>
<tr>
<td>12</td>
<td>Constipated, doesn't move bowels (when not sick)</td>
</tr>
<tr>
<td>13</td>
<td>Cries a lot</td>
</tr>
<tr>
<td>14</td>
<td>Cruel to animals</td>
</tr>
<tr>
<td>15</td>
<td>Delinquent</td>
</tr>
<tr>
<td>16</td>
<td>Demands must be met immediately</td>
</tr>
<tr>
<td>17</td>
<td>Destroys his/her own things</td>
</tr>
<tr>
<td>18</td>
<td>Destroys things belonging to his/her family or other children</td>
</tr>
<tr>
<td>19</td>
<td>Diarrhea or loose bowels (when not sick)</td>
</tr>
<tr>
<td>20</td>
<td>Disobedient</td>
</tr>
<tr>
<td>21</td>
<td>Disturbed by any change in routine</td>
</tr>
<tr>
<td>22</td>
<td>Doesn't want to sleep alone</td>
</tr>
<tr>
<td>23</td>
<td>Doesn't answer when people talk to him/her</td>
</tr>
<tr>
<td>24</td>
<td>Doesn't eat well (describe):</td>
</tr>
<tr>
<td>25</td>
<td>Doesn't get along with other children</td>
</tr>
<tr>
<td>26</td>
<td>Doesn't know how to have fun; acts like a little adult</td>
</tr>
<tr>
<td>27</td>
<td>Doesn't seem to feel guilty after misbehaving</td>
</tr>
<tr>
<td>28</td>
<td>Doesn't want to go out of home</td>
</tr>
<tr>
<td>29</td>
<td>Easily frustrated</td>
</tr>
<tr>
<td>30</td>
<td>Easily jealous</td>
</tr>
<tr>
<td>31</td>
<td>Eats or drinks things that are not food—don't include sweet (describe):</td>
</tr>
<tr>
<td>32</td>
<td>Feeds certain animals, situations, or places (describe):</td>
</tr>
<tr>
<td>33</td>
<td>Feelings are easily hurt</td>
</tr>
<tr>
<td>34</td>
<td>Gets hurt a lot; accident-prone</td>
</tr>
<tr>
<td>35</td>
<td>Gets in many fights</td>
</tr>
<tr>
<td>36</td>
<td>Gets into everything</td>
</tr>
<tr>
<td>37</td>
<td>Gets too upset when separated from parents</td>
</tr>
<tr>
<td>38</td>
<td>Has trouble getting to sleep</td>
</tr>
<tr>
<td>39</td>
<td>Headaches (without medical cause)</td>
</tr>
<tr>
<td>40</td>
<td>Hits others</td>
</tr>
<tr>
<td>41</td>
<td>Holds his/her breath</td>
</tr>
<tr>
<td>42</td>
<td>Hurts animals or people without meaning to</td>
</tr>
<tr>
<td>43</td>
<td>Looks unhappy without good reason</td>
</tr>
<tr>
<td>44</td>
<td>Angry moods</td>
</tr>
<tr>
<td>45</td>
<td>Nausea, feels sick (without medical cause)</td>
</tr>
<tr>
<td>46</td>
<td>Nervous movements or twitching (describe):</td>
</tr>
<tr>
<td>47</td>
<td>Nervous, highspirited, or tense</td>
</tr>
<tr>
<td>48</td>
<td>Nightmares</td>
</tr>
<tr>
<td>49</td>
<td>Overeating</td>
</tr>
<tr>
<td>50</td>
<td>Overfired</td>
</tr>
<tr>
<td>51</td>
<td>Shows panic for no good reason</td>
</tr>
<tr>
<td>52</td>
<td>Painful bowel movements (without medical cause)</td>
</tr>
<tr>
<td>53</td>
<td>Physically attacks people</td>
</tr>
<tr>
<td>54</td>
<td>Picky eater, sick, or other parts of body (describe):</td>
</tr>
</tbody>
</table>

Be sure you have answered all items. Then see other side.
Please print. Be sure to answer all items.

V. 1. About how many close friends does your child have? (Do not include brothers & sisters)
   - None
   - 1
   - 2 or 3
   - 4 or more

   About how many times a week does your child do things with any friends outside of regular school hours?
   - Less than 1
   - 1 or 2
   - 3 or more

VI. Compared to others of his/her age, how well does your child:
   a. Get along with his/her brothers & sisters?
   b. Get along with other kids?
   c. Behave with his/her parents?
   d. Play and work alone?

   Worse  Average  Better
   □  □  □
   □  □  □
   □  □  □
   □  □  □

   Has no brothers or sisters

VII. 1. Performance in academic subjects.  □ Does not attend school because

   Check a box for each subject that child takes
   a. Reading, English, or Language Arts
   b. History or Social Studies
   c. Arithmetic or Math
d. Science
   e. [Blank]
f. [Blank]

c. Other nonacademic subjects

   Falling  Below Average  Average  Above Average
   □  □  □  □
   □  □  □  □
   □  □  □  □
   □  □  □  □
   □  □  □  □
   □  □  □  □

2. Does your child receive special education or remedial services or attend a special class or special school?
   - No
   - Yes—kind of services, class, or school:

3. Has your child repeated any grades?
   - No
   - Yes—grades and reasons:

4. Has your child had any academic or other problems in school?
   - No
   - Yes—please describe:

   When did these problems start?
   Have these problems ended?

   □ No  □ Yes—when?

Does your child have any illness or disability (either physical or mental)?
   - No
   - Yes—please describe:

What concerns you most about your child?

   □ □ describe the best things about your child.

Be sure you answered all items.