Prediction of preterm birth in a twin pregnancy

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Abstract

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Introduction: Twins account for around 2-3% of all births, with trends showing a steady rise in this twinning rate. Twins can be categorised via their chorionicity into dichorionic (DC) and monochorionic (MC) with differing complications. All twins are at risk of preterm birth (PTB) which remains the most significant cause of mortality and morbidity amongst neonates, and twins make up 15-20% of these PTB despite accounting for only 2-3% of all live births. The current evidence for prediction and prevention of PTB in twins is poor and has produced contrasting results.

Methods: Data was collected from Meditech online records, recorded at Liverpool Women's Hospital from 2010-2020. Data collection included maternal characteristics; including BMI, age, parity, ethnicity and data on the twin pregnancy and any previous pregnancy. The chorionicity was collected from fetal software viewpoint. Any fetal loss or stillbirth was identified on Meditech, and further data was collected from viewpoint and scanned notes.

The cohort data was coded and analysed using SPSS software and further split into loss of one or both fetuses. Preterm birth was defined as delivery before 34+0 weeks gestation and analysis was performed on the impact of clinical and demographic factors on gestational week of delivery. Univariable and multivariable analysis was performed to assess the impact of each variable, with multivariable analysis done on all covariates using a backwards step-wise process based on Akaikes information criterion (AIC).

Results: 1584 twin pregnancies were identified from LWH. The chorionicities were 1193 (75.3%) DCDA, 374 (23.6%) MCDA and 17 (1.1%) MCMA. Maternal age (<0.001), white vs non-white ethnicity (0.037), black vs non-black ethnicity (0.012), use of IVF (<0.001), no ART use (<0.001), parity of 0 and 1+ (0.012) and birthweight of twin 1 and twin 2 (<0.001) was found to be significantly different between MC and DC twins.

Spontaneous fetal loss of at least 1 fetus at <24 weeks was higher in MC twins 18 (4.81%) than DC twins 23 (1.93%) (P value 0.002). Loss of one fetus <24 weeks was higher in MC twins 3.5% vs 1.3% p=0.005, but loss of both fetuses was found to be non-significant p=0.141. Relative risk of 1 fetus loss and both fetuses lost was higher in MC twins at 2.76 (CI 1.33, 5.96) and 2.13 (CI 0.76, 5.94).

23.1% of MC and 26.7% of DC pregnancies that lost 1 fetus <24 weeks went on to lose the remaining twin >24 weeks gestation.

Univariable analysis found a reduction in the gestation of the twin pregnancy in pregnancies with a history of previous preterm birth (-1.43 weeks) and a monochorionic chorionicity (-1.82 weeks). An elongation in the gestation of the twin pregnancy was found in a parity of 1 and 2+ (+0.78 and +0.88 weeks) and increasing maternal age (+0.03 weeks for every yearly increase).

Multivariable analysis separated by chorionicity, performed for both a continuous variable and a categorical value, found a found a parity of 1 (+0.94/+0.56 and +1.05/+0.74 weeks) and 2+ (+1.29/+0.87 and +1.24/+1.00 weeks) had a positive effect on both MC and DC pregnancies. History of a previous preterm birth reduced the MC and DC twin pregnancy (-2.00/-1.33 and -2.36/-1.56 weeks). The negative effect of an increasing BMI (-0.03 weeks) was only significant in a MC pregnancy.

Conclusion: Monochorionic twins have higher rates of preterm birth and <24-week pregnancy loss than dichorionic twins. Nulliparity, younger maternal age, history of previous preterm birth, and monochorionicity all reduce the gestational age of the twin pregnancy. The majority of the significant variables have slightly more negative impact on a dichorionic pregnancy than a monochorionic pregnancy. Increasing BMI only had a significant negative effect on a monochorionic pregnancy. This thesis has identified a high-risk twin cohort for preterm birth, which could be used for future research into targeted twin preterm birth prevention studies.

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List of abbreviations

ART – artificial reproductive techniques

BMI – body mass index

BPD – bronchopulmonary dysplasia

CVS – chorionic villus sampling

DCDA – dichorionic diamniotic

DZ – dizygotic

fFN – fetal fibronectin

FSH – follicle stimulating hormone

HFEA - human fertilisation and embryology authority

IVF – in vitro fertilisation

IVH – intraventricular haemorrhage

LWH – Liverpool Women's Hospital

MCDA – monochorionic diamniotic

MCMA – monochorionic monoamniotic

MZ – monozygotic

NICE – National Institute for Health and Care Excellence

NICU – neonatal intensive care unit

PPD – post-partum depression

PTB – preterm birth

sFGR – selective fetal growth restriction

SGA – small for gestational age

TRAP - twin reversed arterial perfusion

TTTS – twin-to-twin transfusion syndrome

TVUS – transvaginal ultrasound

WHO – World Health Organisation

Hypothesis and aims

Hypothesis

This thesis tested the following hypothesis:

1. Twin pregnancies are at high risk of preterm birth, and there are recordable variables that can predict this risk.
2. Monochorionic and dichorionic twins are at different risks of preterm birth and therefore should be treated as two separate populations.

The aims of this thesis are outlined below:

1. Conduct a retrospective cohort study using clinic data from Liverpool Women's hospital to establish the link between recordable pregnancy variables and prediction of twin preterm birth.
2. To further establish the risk of <24-week pregnancy loss in a twin birth, analysed by chorionicity.
3. To potentially identify a high-risk twin pregnancy cohort which can further be used in twin preterm birth intervention research.

Chapter 1 – Challenges of multiple pregnancy and preterm birth

Over the last several decades, there has been an increase in the incidence of multiple births, now accounting for 2-3% of all births (1, 2). Multiple births are any birth that results in more than one child being born, with twins being the most common subset of multiple birth. Twins are more at risk of complications due to being a higher risk pregnancy, they are associated with adverse fetal outcomes; including preterm birth (<37 weeks), low birth weight and stillbirth, contributing to 12% of neonatal deaths (3).

Twins are more likely to be born preterm with nearly 60% of all twins being born before 37 weeks. Despite only making up 2-3% of births, twins disproportionally account for approximately 15-20% of all preterm births (4). Preterm birth is one of the leading causes of mortality and morbidity with studies finding a babies born at 25 weeks' gestation have a 40% mortality rate and 45% morbidity rate (moderate-to-severe handicap) (5). Additionally, twins are associated with 4-fold increase in cerebral palsy and an increase in congenital anomalies when compared with a singleton pregnancy. Babies born from a twin pregnancy are 10 times more likely to be admitted to the neonatal intensive care unit (NICU) when compared their singleton counterparts (6).

1.1 Incidence

In 2019 there were 9,656 multiple births across the whole of the UK, with a multiple birth rate of 15.3 per 1000 pregnancies. In contrast to 20 years previously, the 1999 multiple birth rate was 14.5 per 1000 pregnancies. Despite an overall increase seen in the multiple birth rate, the multiple birth rate has decreased in the last 5 years with a large drop of 3.5% seen most recently from 2018 to 2019 (10,005 to 9,656 births) (7). The rate of monozygotic (MZ) twins has remained fairly stable over the years, however the increase seems to be because of a rise in dizygotic (DZ) twins; thought to be due to increasing maternal age and more widespread access to fertility treatments (8, 9). These factors also interlink as the need for fertility treatments often increases as maternal age increases due to development of factors that predispose to infertility(3). Other less significant factors include family history of twinning, previous twin delivery, diet and maternal height and weight(10), which have been thought to contribute to the increased twinning rate.

1.2 Risk factors for twins

Overall, the incidence of multiple birth has increased in both spontaneous multiple pregnancies and artificial reproductive techniques (ART) multiple pregnancies. A 1999 study conducted in Sweden attributed the increased in multiple birth to in-vitro fertilization (IVF), ovulation induction and increasing maternal age (each of equal weighting). It was also reported that in 1999, in the UK, 31% of live births from IVF were part of a multiple pregnancy (11).

1.2.1 Ethnic groups

Different ethnic groups have shown to have different rates of twin pregnancy, with the highest reported in Nigeria, and lowest in Japan. These twin pregnancies are due to the difference in DZ twins rather than MZ twins. The incidence of MZ twins has been shown to be similar in all ethnic groups throughout the world (12). One study conducted in Nigeria, showed the difference in twinning rates in each region, with western Nigeria having the highest twinning rate of 54.2 per 1000 pregnancies. When split into MZ and DZ, it was shown that the MZ rate across the regions of Nigeria was very similar (4.4-5.1 per 1000 pregnancies), the difference being accounted for by the DZ pregnancies (28.7-49.8 per 1000 pregnancies) (13).

1.2.2 Artificial reproductive techniques

Another reason for increasing multiple births is thought to be artificial reproductive techniques (ART) which have increased over the last several decades. ART are any procedure or medication that assists in achieving pregnancy, and the percentage of new-borns born through such procedures in Europe is thought to be 1.7-2.2% (14). One common complication of ART is multiple pregnancy, and there is current guidance in place to reduce this risk, such as the transfer of one embryo per IVF cycle.

Clomiphene is a follicle stimulating hormone (FSH) up-regulator which was used first line in the medical treatment of polycystic ovary syndrome related infertility. It acts by encouraging follicular development, increasing the chance of multiple follicles becoming mature, increasing the background rate of a twin pregnancy from 1.6% to approximately 5-8% (15, 16). NICE currently recommends that a transvaginal ultrasound be done to check the number and size of the maturing

oocytes to minimise the risk of multiple pregnancy, ensuring that the lowest effective dose of clomiphene is given (17). A 2017 study showed that the frequency of transvaginal ultrasound monitoring during clomiphene use was only 61.45%. One main factor was immediate access to TVUS, showing that clinicians were significantly less likely to follow the current scanning guidance(18). It was previously reported that 8-10% of women taking clomiphene had multiple pregnancies, but a 2020 review has suggested that the rate is much lower at 3-4% when adjusted for certain factors such as BMI and used within the current guidelines (19). Due to the increase in the number of mature follicles being released, clomiphene increases the chance of a DZ dichorionic diamniotic (DCDA) pregnancy. In ovarian stimulation, it is more difficult to control the number of mature oocytes that develop and therefore the number of potential embryos that implant.

The human fertilisation and embryology authority (HFEA) introduced a two-embryo policy that came into effect in 2004, stating only two embryos can be transferred into a woman under the age of 40 in each IVF cycle. In 2010 only 14.9% of IVF treatment cycles had one embryo transferred (20). The birth rates reported in a 2005 trial showed when transferring one or two embryos in a cycle, the live birth rates were 38.8% and 42.9% respectively, with the twin pregnancy rate being 0.8% and 33.1% (21), showing a minimal difference in live birth rate but a large reduction in the risk of twin pregnancy. More recently in 2008, it was reported by the European society for human reproduction and embryology that 22% of all assisted reproductive deliveries were multiple births (22).

Transferring just one fertilised embryo in the IVF process will not prevent the embryo from splitting to create a MZ twin pregnancy. Studies have reported a 1.5-3% MZ twin live birth rate in IVF pregnancies compared to 0.4-0.45% rate reported in natural conception (23-25). A link between younger females (<35 years) and transferring blastocysts has been shown to be statically significant to increase the risk of a MZ pregnancy in a 2019 study (26). Although studies have shown an increased rate of MZ twins in IVF pregnancies (27) it has been stated that larger cohort studies are needed to assess the full relationship and risk IVF poses. Despite a reduction in twin pregnancy overall after IVF in the last 20 years, the incidence of monochorionic twins was found to have increased from 1.6% 2004-2005 to 2.5% in 2009-2010 after IVF (28).

Although transferring one embryo rather than two in an IVF cycle as shown to be effective for reducing the risk of twin whilst only slightly impacting the chance of a live birth, single embryo transfers are still rare due to the financial and cost effectiveness of the procedure (29). Multiple studies have found comparable fetal and maternal outcomes in ART twin pregnancies when

compared to spontaneous twin pregnancies. In Finland where the transfer of just embryo is recommended, it has been found that the twin rate has been lowered to less than 10% (30).

1.2.3 Maternal age

In 2019, the mean age of a mother at childbirth was 30.7 years. This is an increase of approximately 16.3% from 1973 when the mean age was 26.4 years. The rate of childbirth in women over 40 years is now higher than the fertility rate of women under 20 years, a trend last seen in 1947 (31). It was also reported in the 2019 data that in the age groups 40-44 and 45+, the multiple birth rate was 24.6 and 73.2 per 1000 maternities. This is in contrast to a multiple birth rate of 15.9 and 19.1 per 1000 maternities in 30-34 and 35-39 years. In 1999, 20 years previously, the multiple birth rate per 1000 maternities in the 45+ age group was 47.4 (31) displaying an 54.5% increase in this age group. Several studies have reported that the rate of a twin pregnancy has been shown to increase with maternal ages 35-39 years. As with the difference in ethnic groups, this increase is due to dizygotic twin pregnancies as opposed to monozygotic pregnancies. ART and egg donation have resulted in more women being able to have children in their penultimate childbearing years (32), and therefore increased twin pregnancy in women over 35 years. This comes with its own challenges that are not only associated with a multiple pregnancy but with an older mother.

1.3 Twin Classification (chorionicity and amnionicity)

Twins are classified by their chorionicity and amnionicity. They are either dichorionic diamniotic (DCDA), monochorionic diamniotic (MCDA) or monochorionic monoamniotic (MCMA). They can also be separated by their zygoticity, monozygotic; from one fertilised embryo, or dizygotic; from two fertilised embryos.

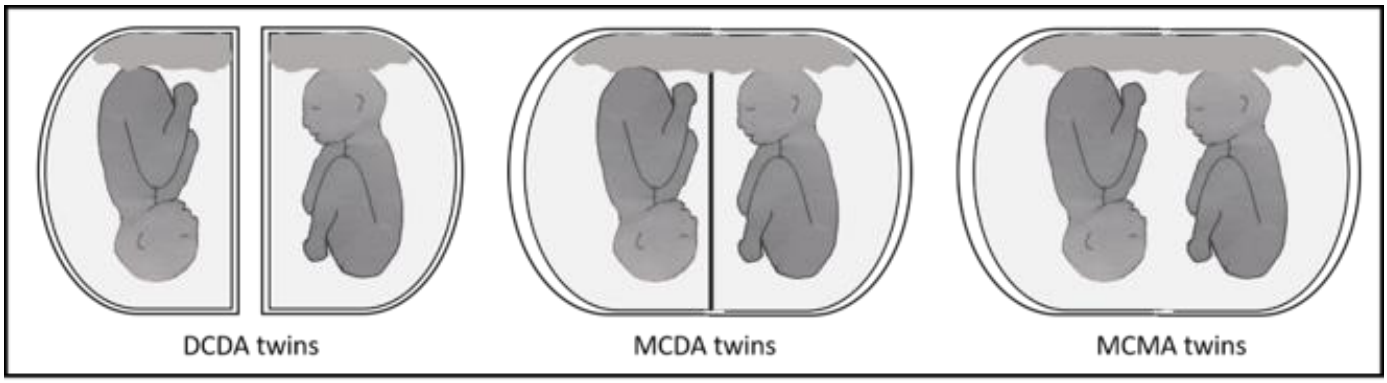


Figure 1 - Chorionicity of a twin pregnancy

Chorionicity can be defined as monochorionic (sharing one chorion) or dichorionic (separate chorions). The chorion refers to outer membrane surrounding the fetus containing the amnion membrane, the chorion contributes to placental development resulting in each chorionic membrane being connected to a single placenta. The amnion is a thinner membrane inside the chorion that directly contains the amniotic fluid and fetus.

In a DC twin pregnancy, the fetus' have their own separate chorionic membrane, thus are also diamniotic and have their own separate placentas. A MCDA pregnancy will have one chorionic membrane with two amniotic membranes. This results in both fetus' sharing one common placenta but being contained in their own amniotic sac with dividing membranes. A MCMA pregnancy results in the fetuses being within the same chorionic membrane and amniotic membrane, sharing both a placenta and amniotic sac.

All twins born from two embryos (DZ) develop separately within their own chorion resulting in DCDA twins. However, MZ twins (from one embryo) can be any of the three chorionicitys. It is thought that approximately 33% of MZ twins are DCDA, 65% are MCDA, with the remaining 2% being MCMA (33). This distinction is due to the timing of the embryonic cleavage, with the earliest cleavage resulting in DCDA twins (before 3 days conception) (34), later MCDA and the very late cleavage around 8 days after fertilisation (35) resulting in MCMA twins respectively. Separation after day 13 usually results in conjoined twins due to the incomplete separation (36)

Chorionicity is determined during prenatal ultrasound scanning, with zygosity (number of fertilised ovum) able to be predicted in approximately 65% of pregnancies (37). Chorionicity can be recorded effectively from a gestation of 7 weeks by using transvaginal ultrasound (38).

DCDA pregnancies are identified by the presence of two separate placentas and a thick membrane showing two separate gestational sacs. In some pregnancies only one fused placenta can be seen, making it difficult to determine the chorionicity. In these cases, the absence of chorionic tissue into the intertwin membrane implies a MC pregnancy (39). The presence of chorionic tissue would result in a Lambda sign on ultrasound indicating a dichorionic pregnancy (40). Additionally, a thick membrane that measures >2mm is a positive predictor for a DCDA pregnancy in 92% of cases (41). The fetuses are separated by three layers; a thick layer of chorion with the two separate amniotic sac membranes on either side (42).

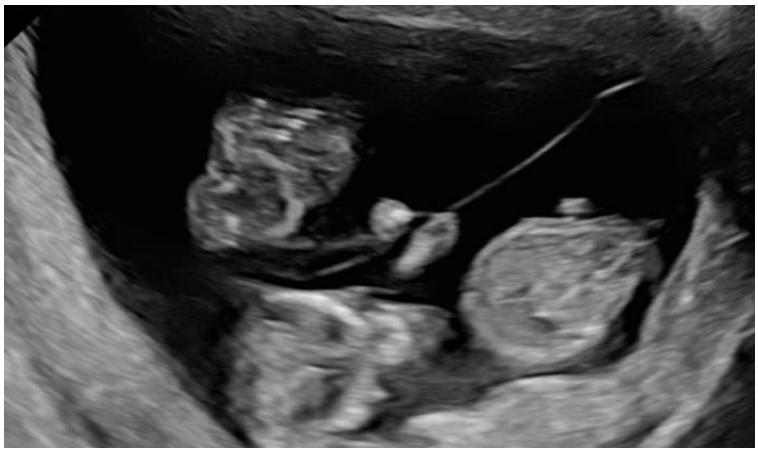


Figure 3 - MCDA twin pregnancy

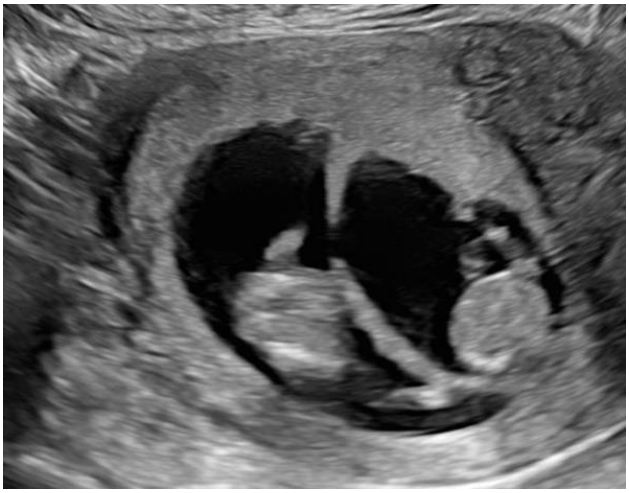


Figure 2 - DCDA Pregnancy

A MCDA pregnancy will show two separate layers; the two amniotic sac membranes, with the absence of the thick layer of chorion (42). A thin membrane, one placenta and two separate gestational sacs can also be used to indicate a MCDA pregnancy (36). The T sign on ultrasound (two thin amniotic sacs bound together) indicates the diagnosis of a MCDA twin pregnancy with sensitivity of 100% (43). The T sign will not be seen in a MCMA pregnancy due to the lack of separating amniotic sacs. The number of placenta masses seen on ultrasound can occasionally be misleading due to fusing of the placentas in a DCDA pregnancy or a bilobed placenta in a monochorionic pregnancy (44).

It is harder to determine the chorionicity of a pregnancy the more advanced it is. The dividing membrane that is very apparent in the first trimester in DCDA twins is less visible as the pregnancy progresses, as it becomes difficult to distinguish between the thick chorionic layer and the thin amniotic sac membranes. The best way to determine chorionicity has showed to be an ultrasound within the first or the early second trimester (39). At a later gestation, discordant sex of the twins is the most obvious sign of a DCDA pregnancy (36), although structural abnormalities can arise in the fetus' meaning this is not the most reliable method. Some MCDA pregnancies can become iatrogenic MCMA through interventions, particularly for vascular complications (45), so it is important to determine the chorionicity as early as possible before these interventions are indicated.

1.4 Twin specific complications

MC twins are associated with a higher risk of adverse outcomes when compared to DC twins. Therefore, it is important to determine the chorionicity of a pregnancy due to the increased risks associated with monochorionicity. Accuracy is improved when the assessment is undertaken at 14 weeks or less, with some results after 14 weeks having shown to pregnancy being labelled as the incorrect chorionicity in up to 14% of cases (44).

Two ultrasound screening studies showed a spontaneous death of at least one fetus between 10/14-24 weeks in 12% in monochorionic twins and 2.5% in dichorionic twins (46, 47). MC twins are higher risk due to their shared circulations through vascular anastomoses, this single placenta can lead to problems unique to monochorionic twin pregnancies such as twin anemia-polycythemia sequence (TAPS), twin-to-twin transfusion syndrome (TTTS), and death or risk of neurological damage in one twin if the other twin dies during the pregnancy. Other complications that occur in singleton or DCDA twin pregnancies, such as growth restriction (or selective fetal growth restriction sFGR) and fetal abnormalities, are also heightened due to the connection between the twins and therefore their reliance on one another. In one analysis from a single centre taken over 11 years, the overall mortality rate for MCDA twins was 10% (42).

MCDA twins have a mortality rates close to twice as high as DC twins, and four times as high when compared to a singleton pregnancy. Many MC twin loses occur before 24 weeks, meaning perinatal mortality data often underestimates the loss of MC twins. Most of these earlier loses are thought to be due to TTTS (46).

1.4.1 Twin-to-twin transfusion syndrome

TTTS occurs only in MC twins due to the shared placental circulation, the vascular connections allow a gradual shift of blood from one twin to the other (donor to the recipient). It is estimated that it occurs in 9-15% of MCDA twin pregnancies (48, 49), with the onset usually between 16 and 26 weeks (50). The prognosis in a TTTS pregnancy is overall worse for the donor twin with a prenatal death rate of 18-35% (51, 52).

The transfusion imbalance across the placenta creates a donor and a recipient twin. Characteristics such as polyuria, polyhydramnios and hypervolemia are seen in the recipient, with oliguria, oligo-anhydramnios and hypovolemia being seen in the donor (53).

Without treatment, there is a significant rate of mortality for TTTS, with 100% mortality for the most advanced stage. Definitive treatment involves an intrauterine fetoscopic laser photocoagulation of the common vascular anastomoses in the placenta (54). The choice to perform invasive treatment in the management of TTTS depends on the severity of the disease. Severity can be graded into four or five stages, often known as the Quintero stages with the fifth stage generally being the death of one fetus.

Stage	**Characteristics** (55)
1	Poly/oligohydramnios
2	As above + absent bladder in the donor twin
3	As above + at least one of 1) umbilical artery absent or reversed end-diastolic velocity, 2) Reverse flow in the ductus venosus, 3) pulsatile venous flow
4	As above + hydrops
5	As above + demise of at least one twin

Table 1 Quintero stages for TTTS

Stage 1, defined as a discordance in the amniotic fluid level, is generally treated conservatively as a previous study has shown that 70% of these cases do not progress past this stage (56). A more recent randomised control trial reported contrasting results with only 41% of stage 1 TTTS not progressing to a more severe stage. Despite this, the RCT still recommended expectant management as a reasonable option for stage 1 TTTS, with intervention only recommended if the TTTS progressed to stage 2 or more (57).

In cases where the fluid discordance is more severe, intervention is necessary to prevent fetal mortality and/or morbidity. In the less severe stages, treatment can involve amniodrainage. This aims to reduce the fluid level in a recipient twins polyhydramnios (58), whilst simultaneously decreasing intrauterine pressure with the hopes a preventing preterm onset of labour (59).

The most severe TTTS is treated by endoscopic laser coagulation. Unlike surveillance or amniodrainage, laser coagulation treats the direct cause of TTTS. It works by directing a large amount of energy towards fetal vessels until blood flow is prevented, aiming to create a similar environment to dichorionic placentas. By severing the common anastomoses between the two fetuses, it is hoped that the blood flow is more equally distributed (60). 10% of all treated

pregnancies after the procedure result in the rupture of membranes but other complications are uncommon (61). The survival rate after endoscopic laser therapy for at least one fetus was shown to be 76% in one multicentre study versus a 51% survival rate in amniodrainage alone (62). Endoscopic laser is the most appropriate treatment for severe TTTS before 26 weeks gestation, but is not performed at stage 5 due to the death of one twin. The laser treatment shows the most benefit in TTTS stages 2-4, with previous studies showing only a small reduction in fetal loss in treatment of stage 1 TTTS vs expectant management (loss of both twins 13.2% vs 15.1%) (63).

1.4.2 MCMA pregnancies

Cord entanglement is a complication that occurs in MCMA pregnancies due to the sharing of the placenta, amniotic sac and lack of separating membrane or septum (64). Up to 71% of MCMA pregnancies have cord entanglement with more than 50% of mortalities due to this complication (65). NICE states that MCMA pregnancies should be delivered between 32-33+6 weeks (66) due to this complication. The late cleavage of the embryo, approximately eight days post fertilisation, also increases the risk of asymmetrical placenta distribution, leading to birth weight discordance. TTTS has been recorded to occur less frequently in MCMA pregnancies (3%) (67) as opposed to MCDA pregnancies (9-15%).

1.4.3 Birth weight discordance

Birth weight discordance is the percentage difference the weight of both babies in a pregnancy. Both DC and MC twin pregnancies can have birth weight discordance of differing severities. In approximately 7-11% of MCDA twin pregnancies, there is a birth weight discordance of more than 25% (68, 69).

Severe birthweight discordance is considered severe at higher than 25%, whilst 15-24% indicates a mild discordance (70). A high birth weight discordance is an important indicator in the mortality and morbidity in MC twin pregnancies. In MCDA twin pregnancies, a common cause of a large birth weight discordance is TTTS. TTTS creates a donor and a recipient twin, also leading to a discordance in amniotic fluid distribution (polyhydramnios/oligohydramnios) (71).

Isolated birth weight discordance is seen in both DCDA and MCDA pregnancies. Some DCDA pregnancies are DZ, the birth weight discordance could be explained due to difference in genetic

factors. All MCDA pregnancies are MZ, in these cases birth weight discordance in the absence of TTTS cannot be explained due to genetic factors. Unequal placental sharing in a MCDA pregnancy is the most likely cause of a significant birth weight discordance (72). The placental distribution indicates the direct sharing of circulation that each individual twin has with the mother, and therefore has a direct effect on growth in each individual fetus. Even in the absence of TTTS there can be amniotic fluid balance discordance, due to the smaller twin naturally producing less urine than the larger twin. In cases such as these, TTTS can be ruled out with the absence of polyhydramnios in the larger twin (42).

MC pregnancies also have increased risk to the mother including threatened preterm labour, gestational diabetes, and TORCH infections. Although this increased risk is also present in DC twin pregnancies when compared to singleton pregnancy, the risk is higher in the MC twins (73). However, the risk of pre-eclampsia has found to be increased more in DC pregnancies than MC in a recent review (74), largely due to the presence of increased placental mass which heightens risk (75)

DCDA twins who are MZ have birth weight discordance comparable to DZ twins , implying chorionicity over zygosity is the predominant factor in the discordance (76). It has been shown in certain studies that the larger the combined birthweight of the twins, the less likely they will have a significant birth weight discordance. This has been suggested to be due to twins with large weight discordances being induced therefore delivered early. It has also been suggested that if a uterus can nurture the pregnancy for longer, it may be more efficient in providing for two babies equally (77).

Both TTTS and sFGR can happen simultaneously in MC twins. FGR is defined as a fetus having an estimated fetal weight of below the 5^{th} or 10^{th} percentile for its gestational age. sFGR is defined as one twin the pregnancy being classed as FGR whilst the other twin is not FGR. sFGR occurs in around 12.5-36% of all MC pregnancies, although this number is not clear and may include cases of TTTS (49, 52, 78, 79). It has been previously suggested that FGR twins have a better outcome than a FGR singleton born at the same gestation due to twin having an accelerated pulmonary maturation but this theory has not been proven with multiple studies finding similar prognosis between singletons and multiples (80).

Birthweight is one of the strongest predictors for disability and perinatal mortality, in both singleton and multiple pregnancies. The mean birthweight for twins remains similar to that of a singleton until around 32 weeks' gestation. After this, growth slows in a twin pregnancy until term (81). On average twins weigh around 600g less than singletons born at the same gestation (82). Multiparous women

have, on average, twins of larger combined birth weight when compared to twins of primiparous women (83).

It has been reported by several investigators that, on average, male twins are heavier than their female twin in DZ pairs, and also heavier than similar DZ female pairs (84). This is despite female twin on average having a longer gestation than their male counterparts, with female same sex twins having similar average gestation to female-male twins. It has also been suggested that in male-female DCDA twins, the female fetus prolongs the gestation of the pregnancy past that which would be expected in a similar male-male DCDA twin set (85) after being adjusted for gestation. The birth weight discordance between male and female twins, where the male twin is heavier, has been shown to more statically significant in the later gestations or when the weight of the twins was above 3000g. It has been suggested that the inter-sex differences come into play at a later gestation age due to the effects of fetal testosterone resulting in an increased body mass (86).

1.4.3 Placenta

Abnormal cord insertions are also seen more frequently in twin pregnancies, in 3-17% DC and 10-45% MC placentas. Other abnormalities such as a singular umbilical artery has shown to occur three to four times more frequently in twin pregnancies than in a singleton pregnancy (87).
Birthweight in twins has shown to be significantly lower in abnormal or peripheral umbilical cord insertion when compared to central cord insertion. Central cord insertion is also found less frequently in MC twins (55% males, 52% females) when compared to DC twins, more in MZ DC twins (67% males, 61% females) and highest in DZ twins (81% males, 82% females). When comparing peripheral cord insertion in both DZ and MZ pregnancies, DZ twins weighed significantly more (88).

In some cases, the placentas in a DCDA pregnancy can be fused, both in MZ and DZ pregnancies. The fusion has not been shown to significantly affect the birth weight of the babies born in a DZ pregnancy, but has shown to have a more significant negative affect on the birthweight of MZ DC twins (88, 89). It has been speculated that one cause of such discrepancy between the placenta distribution in MZ DC twins is the vascular supply in the implantation site. It is thought that the placentas may fuse sooner due migration to better vascular supply in one area (88) and this may explain the finding of more peripheral cord insertion when compared to DZ twin placentas that have fused.

1.5 Pregnancy complications

Congenital anomalies in twins have shown to be higher than in singleton pregnancies. A study in Europe showed that congenital anomalies were 27% higher in multiple births than in singleton births. There was also a 70% rise in congenital anomalies in twin pregnancies over a period of 23 years (90), this paired with the rise in twin pregnancies being attributed to DC pregnancies, displays that the congenital anomalies are not only associated with MC pregnancies. In discordant cases of congenital anomaly, termination of the affected fetus is less likely than in a singleton pregnancy due to the effect on the unaffected co-twin. This could account for the higher numbers of DC twin congenital anomalies seen, as many singleton congenital anomalies may have been terminated.

Concordant anomalies have been shown to be higher in MZ twins than DZ twins, but only certain systems such as nervous, circulatory and cleft/lip/palate showed a significant difference (91). These such anomalies have shown previously to have strong genetic associations over environmental factors such as sharing a uterus.

MZ twins are from a single zygote and therefore have an identical genome. This could suggest that all MZ twins would be concurrent for congenital anomalies. The most common congenital anomaly in MZ twins has been reported to be congenital heart defects with 44.4% being represented by this group in a 2019 study. There are several theories about the explanation of discordance of congenital abnormalities in a MZ twin pair. Such theories include abnormal mitosis after the splitting of the embryo, unequal correction of an initial aneuploid zygote, gene mutation, or difference in gene expression (92).

Twin pregnancies as a whole are considered a high-risk pregnancy. Many pregnancies are delivered early to prevent the risk of intrauterine death or stillbirth. When compared to singleton pregnancies, the stillbirth risk is increased 5x in DC and 13x in MC pregnancies. The optimal gestation for a twin pregnancy, to both reduce the risk of stillbirth and the risk of neonatal complications, is unknown. The current guidance suggests 37-39 weeks in a DC and 34-37 weeks in a MC pregnancy. A 2016 review signified that the risk between stillbirth and adverse neonatal outcomes is balanced up to 37+0-6 in DC and 36+0-6 in MC pregnancies, at these points the stillbirth risk rises about the risk of neonatal death (clear evidence only shown in dichorionic pregnancies) (93).

Preterm twin pregnancy has been shown to have worse infant morbidity and mortality in comparison to a singleton pregnancy at the same gestation. One study found that twins were

significantly more likely to have a severe (grade III/IV) intraventricular haemorrhage (IVH) than a singleton of the same gestation (94) , with the neonatal death in babies born at <29 weeks being higher in twins than singletons (95). Other neonatal complications include respiratory distress syndrome, retinopathy of prematurity, necrotising enterocolitis and oxygen requirement after birth. A 2021 study displayed that the frequency of adverse neonatal outcomes is similar in both spontaneous and iatrogenic preterm births (mix of singleton and multiple pregnancies), with the exception of necrotising enterocolitis being more frequent in the iatrogenic preterm infants (96).

1.5 Preterm birth

Preterm birth (PTB) is defined as birth before 37 weeks of gestation (97), with further classification into 34 to 36+6 weeks as late preterm birth and <34+0 weeks as early PTB (98). Early PTB can also be subdivided into extreme preterm (<28 weeks) and very preterm (28-32 weeks) (99). PTB can occur spontaneously or can be the result of iatrogenic interventions, either for the life of the fetus or the life of the mother. It is thought that 40-50% of PTBs are spontaneous, with 25-40% caused by the premature rupture of membranes, leaving 20-25% iatrogenic due to medically indicated reasons (97). The neonatal mortality and morbidity are at the highest risk in early PTB with the risk reducing as gestational age increases. It is thought about 40% of twin births occur in the late pre-term stage of 34-36+6 weeks (100) which has shown to have negative effects on respiratory morbidity (101) long term development outcome and cognitive skills (102) when compared to a baby born at term. Across the world, PTB is the most common cause of neonatal death, with only 10-50% of neonates born at a gestation of 24 weeks surviving the first 28 days of life (103). Babies born pre-term have a higher risk of health issues in both the short and long term. PTB as a whole is responsible for 70% of neonatal mortality and 75% of neonatal morbidity (104).

For both singleton and multiple pregnancies, PTB is associated with increased risk of neonatal death and neonatal complications including neurodevelopment conditions such as cerebral palsy (105). The prevalence of cerebral palsy is reported to be around 14% in babies born 22-27 weeks, with it decreasing to <1% by 32-36 weeks gestational age (106). Other neurological deficits such as attention deficit hyperactivity disorder (ADHD), cognitive impairment, hearing impairment and visual impairment have all be reported to higher in children born at lower gestation age (107-110). Very pre-term children have been reported to have lower cognitive scores than their peers, with this deficit continuing into adulthood, achieving lower IQ scores (111).

PTB has also been shown to have effects later on in childhood with differences in development able to be seen in seemingly 'unaffected healthy' preterm children. One study reported pre-term children aged 6-9, that had no reportable health deficits, performed less well in certain visual-perceptual tasks in comparison to children born at term (112).

Another study looked at disease burden in children aged 3 and 5 years according to their gestational age at birth. Children born at both <32 weeks and 32-33+6 weeks gestation were shown at both aged 3 and 5 to be more likely to be classified as underweight in comparison to a child born at full term gestation. This study consistently showed that an increasing gestational age decreased adverse outcomes including growth, longstanding illness and asthma (113). It has been reported that 86% of children with severe disabilities at 30 months of age, still have moderate to severe disability at 6 years (114).

Bronchopulmonary dysplasia (BPD) is a form of chronic lung disease associated with PTB and can lead to short and long-term morbidity and mortality. It is thought that 35-45% of babies born before 28 weeks' gestation develop BPD (115), with severe disease severity able to be predicted by oxygen dependence at 36 weeks of age (116). A 2014 study reported that pre-term children with a previous diagnosis of BPD had significantly lower lung function compared to children born pre-term without a previous diagnosis of BPD when tested at aged 9 (117).

1.5.1 Family implications

PTB has shown to have a psychological impact on the parents and other family members. Parents are separated from any babies admitted into the NICU and potentially have to witness distressing and life threating medical complication (118). Parents may also be separated due to other childcare commitments and other aspects of family life. Rate of anxiety and depression have reported to be 2-5 times higher in the mothers of pre-term infants (119). Additionally, the mother suffering post-traumatic stress symptoms has also shown to be increased in mothers of pre-term infants compared to term infants (34% Vs 4%) (120). A systematic review reported a post-partum depression (PPD) rate of up to 40% in mothers with pre-term infants, with the average incidence PPD rate being 10-15% (121). Studies have also shown a similar trend with fathers of pre-term infants developing depression, although a less significant difference was reported when compared to mothers (122).

1.5.2 Cost

An increase in morbidity rate in pre-term infants has increased demands on the health service, especially related to the economic and financial costs. Due to the long-term health effects of PTB, the financial costs increase well past childhood, but studies have shown that the hospital inpatient costs straight after birth are responsible for 64-92% of costs per pre-term infant (123), with a large amount of the remaining costs being attributed to the following 2 years following birth (124). Conditions such as BPD, found most commonly in pre-term infants, often cause at least one hospital admission in the first year of a baby's life with some requiring admission to the intensive care unit (125). These conditions that cause long-term health issues add to the financial cost of a pre-term infant but also further into their childhood and beyond. A UK study estimated that a pre-term child cost £22,885 more if they survived to the age of 18 when compared with a child born at term, with an extremely pre-term child costing significantly more (123). Pre-term children have shown to have increased costs in varying services including inpatient care, outpatient care, education and social care (126).

1.6 Cause of twin preterm birth

The pathophysiology of spontaneous PTB in twins is largely unknown, it is thought to be due to various different factors and differs from singleton pregnancies. Different variables including cervical insufficiency, stress, uterine stretch and infection have all been suggested with these being increased in a twin pregnancy due to larger fetal mass and amniotic fluid volume (127).

Risk factors for pre-term labour and birth are largely unknown in a twin gestation. Certain studies have suggested a previous PTB increases the risk of a spontaneous pre-term twin birth (128, 129). Other maternal factors that have shown to increase the chance of a PTB are maternal age less than 20 years, obese BMI or a nulliparous mother. Early term births (34-36+6 weeks) have been shown to be increased in mothers who are non-white race, smokers or have diabetes in pregnancy (98), but this is inconsistent across reviews (130).

1.6.1 Fetal fibronectin

A 1996 study looked at predictors of PTB in twin pregnancies. It looked at various factors including cervical length at certain gestational weeks, fetal fibronectin levels every 2 weeks and presence of bacterial vaginosis, the outcomes were reported via spontaneous preterm birth at <32, <35 and <37

weeks gestation. Significant predictors of PTB found were a short cervix (<25mm) measured at 24 weeks, a positive fetal fibronectin result measured at 28 weeks resulted in a <32-week PTB. This study concluded that most known risk factors for spontaneous preterm singleton birth were not significantly associated with twin PTB (131). A large systematic review showed a significant link between a positive fetal fibronectin (>50 ng/mL) and subsequent preterm twin birth. This was shown to be significant at a large range of gestations (<28-<37 weeks) (132).

1.6.2 Previous preterm birth

A large 2013 study concluded that having a previous preterm singleton pregnancy was a significant risk factor for a subsequent preterm twin pregnancy (133). This study excluded any pregnancies affected by twin-to-twin transfusion syndrome and for iatrogenic delivery reasons not found in both pregnancies. Although not specifically separating the pregnancies via chorionicity, this method would have excluded many MC pregnancies, leaving DC and uncomplicated MC. This study also separately defined preterm birth as <37 and also <34 weeks. A 2007 study found similar conclusions when looking at previous PTB, although this study defined the twin preterm birth as <37 weeks (128).

1.6.3 Maternal age

The effects of maternal age on preterm twin birth has been reported in various studies. A 2015 study looked at singleton and twin births and found mothers aged <30 years were at significant risk of PTB for both singleton and twin pregnancies, with it being more significant amongst twin pregnancies. This same study also reported that women >35 years were not at any increased risk of preterm twin birth. This study however only looked at twin births conceived via in vitro fertilization (134) and did not separate the twins by chorionicity. This study also reported preterm birth separately as <37, <32 and <28 weeks gestation and no significant difference was found in these preterm rates amongst women 30-34, 35-39 and ≥40 years. Another study that again only looked at twins born after the use of ART also reported no significant incidence of twin PTB in older mothers (>40 years). This study only included nulliparous women, and made a distinction between chorionicity by only including DCDA twin pregnancies (135).

1.6.4 Artificial reproductive techniques

A 2009 study looked into the effect of parity of gestation and found a significant difference in the prematurity rates between nulliparous and multiparous women with nulliparous women being at a higher risk of having a preterm twin birth. However, this study only enrolled women who had achieved a twin pregnancy after ART (136). A 2008 study found that twin pregnancy conceived via ART were less likely to be born very preterm when compared to spontaneously conceived twin pregnancies. This difference was only reported in nulliparous women and not seen in multiparous women (137).

1.6.5 Twin sex

A 2013 review looked at twin sex and the risk of PTB. When comparing same sex twins, male sex was found to be a significant risk factor for PTB in twins, including both early term birth and very early preterm birth. In this same study, maternal age, multiparity, BMI and ART did not show to be significant risk factors (138). This finding has been shown across multiple different studies with findings of male-male twin pairs having the highest PTB rate, female-female pairs lower and discordant sex twin pairs the lowest rate of PTB (139). This study included 148,234 twin pairs, however it did not separate these by chorionicity. A 2001 review separated further and looked at only DZ twin pairs, this found that the gestation of discordant sex twins and female twin pairs was similar but the gestation of male-male pairs was found to be significant shorter than the former two (140).

1.6.6 Smoking

Women who smoke during their twin pregnancy have found to have a shorter gestation than non-smoking twin mothers. A 2001 study found that this persisted even when other factors such as maternal age, BMI, alcohol intake, marital status, education, occupation, previous preterm birth and ART were adjusted for (141). This study did not report on twin chorionicity and therefore didn't adjust for this factor.

1.7 Conclusion

Overall, the current literature shows some significant risk factors for PTB in twin pregnancy including previous preterm birth, nulliparous mother and obese BMI. However, the majority of this literature has reported preterm birth in twins as <37 weeks gestation, despite current guidelines recommending both MC and DC to be induced before or at this gestation. There is a need for risk factors for twin PTB to be reported for earlier gestation, such as <34 weeks.

The current literature also highlights the lack of reviews and studies reporting their results by chorionicity, as a lot of current literature reports the MC and DC as one overall population, or only reports data on DC. It is known that MC twins are delivered earlier and have more adverse outcomes than DC twins, so it could be suggested that PTB risk factors could be more significant or different between the two chorionicitys.

Chapter 2: Incidence of pregnancy loss at <24-week gestation in a twin pregnancy

2.1 Introduction

The incidence of twin pregnancies has increased over the last couple of decades now accounting for approximately 2.09% of all live births(142). Twins have higher complication rates than their singleton counterparts with chorionicity playing a major role in the outcome of the pregnancy. Mortality and morbidity are greater in a twin pregnancy when compared to singleton pregnancies, with low birth weight, perinatal and infant deaths all showing an increased relative risk of at least 2.75 (142).

Multiple pregnancy is also a major risk factor for stillbirth (143). A 2016 review suggested that due to the increased stillbirth risk in a twin pregnancy, uncomplicated monochorionic (MC) and dichorionic (DC) pregnancies should be delivered at 36 and 37 weeks' gestation respectively (144, 145). This data alone shows a higher level of surveillance is needed in twin pregnancies, regardless of their chorionicity.

MC twins have been shown to have higher rates of adverse outcomes that their DC counterparts. This is due to conditions such as selective fetal growth restriction (sFGR) twin-to-twin transfusion syndrome (TTTS), twin reversed arterial perfusion (TRAP) syndrome and rarely conjoined twins (146). A large amount of these MC conditions are due to vascular complications, each MC placenta has its own different vascular composition and therefore can have a variety of vascular complications(147).

The aim of this study is to find and report any differences in maternal and pregnancy variables between MC and DC twins. It will also compare the risk of miscarriage rates before 24 weeks between MC and DC twin pregnancies, and to see if the risk of losing one fetus or both fetuses is different between the two chorionicitys.

2.2 Methods

This retrospective cohort study included all women who gave birth to twins between January 2010 and November 2020. The electronic database (Meditech) was searched to identify every twin pregnancy who had their dating scan and delivered at Liverpool Women's hospital (LWH) between

January 2010 and November 2020. Any women who consequently delivered twins at LWH who had not initially had their antenatal care at LWH were not included in this study. This included women who moved locations later in their pregnancy and women who delivered at LWH due to high risk pregnancies but initially had their pregnancy care in a different trust. These twin pregnancies were identified by two fetuses being reported on an ultrasound report and entered into the online dataset as a twin pregnancy. Any higher order multiple pregnancies were excluded from this study, including any pregnancies that became a two fetus pregnancy through fetal loss or selective reduction.

Baseline maternal characteristic data including BMI, age, race and smoking status were collected from the booking history using Meditech online records. This also included collecting information on any artificial reproductive techniques used to achieve the current twin pregnancy. Obstetric history was also collected from Meditech's previous delivery summaries, this included previous mode of delivery on the immediate previous pregnancy and additional data on any previous birth before 37 weeks gestation.

Data on the twin pregnancy was collected from the delivery summary. This included indication for delivery, gestation at delivery, birth weight of each twin and live or still birth status of each fetus. The chorionicity of each pregnancy was identified from specialist fetal medicine software (Viewpoint) where it was reported as DCDA, MCDA or MCMA. Chorionicity is determined at 10-13+6 weeks on ultrasound by the presence of lambda or T signs. If chorionicity cannot be determined then discordance sex of the twins can be used or number of placental masses found on ultrasound. If the chorionicity cannot be determined by these methods, then the pregnancy is treated as monochorionic.

Gestation of the pregnancy was determined between 10-13+6 weeks on the mother's first ultrasound. Crown rump length was measured and the measurement of the larger twin was taken. If this measurement was found to be >84mm, then head circumference was used instead to determine gestational weeks (148). The larger twin's measurement is used to prevent any early onset growth pathology in the smaller twin affecting the gestational week determination. This data was collected from the delivery summary on Meditech.

Miscarriage data was identified from a delivery summary reporting any twin born without signs of life or stillbirth of one or both fetus. Viewpoint was then searched to find the gestation of miscarriage, either through reporting miscarriage of whole pregnancy or in ability to find one twin

heartbeat on examination. Any unclear data was then further reviewed using scanned notes to identify the gestational age of the fetal loss. The data were then reported by chorionicity and one or both fetus lost before 24 weeks gestation.

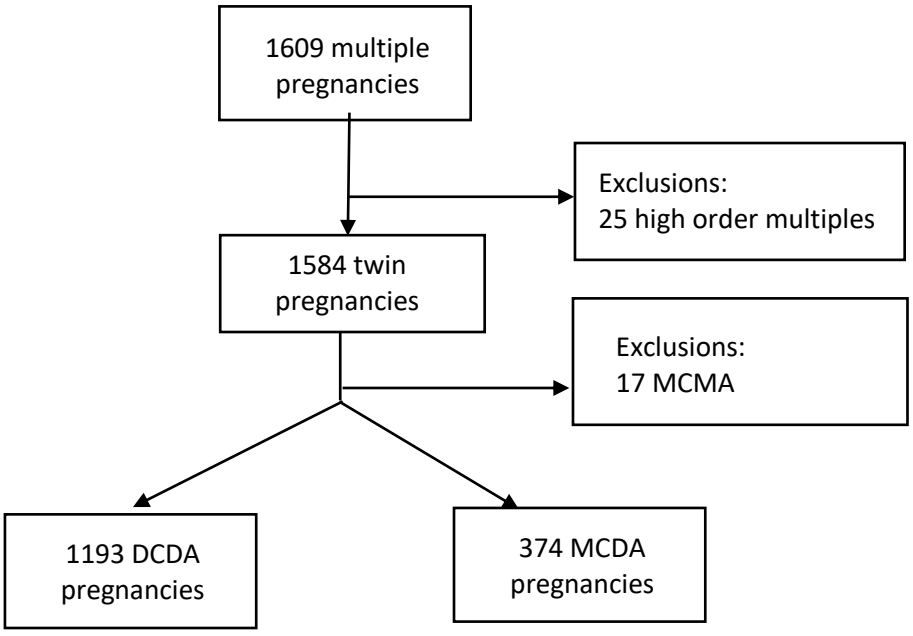
Data was coded and analysed using the statistical software package SPSS. Categorical variables were reported by their frequencies and proportions, with the chi-squared test used to compare MC and DC pregnancies, and assess statistical significance. Continuous variables were reported by their means plus standard deviations and the independent t-test was used to find their statistical significance. A p value of 0.05 was used to report statistical significance. The data were further analysed to determine the risk of miscarriage in a twin pregnancy and the relative risk between chronicity's. This analysis was further split into loss of one or both fetus.

2.3 Results

2.3.1 Twin Cohort

1584 women with twin pregnancies, booked and delivered at LWH, were identified between January 2010 and November 2020 that fulfilled the inclusion criteria (25 higher order multiple pregnancies were excluded from this). Of these 1193 (75.3%) were DCDA, 374 (23.6%) were MCDA and 17 (1.1%) were MCMA. For the purpose of this study, all MCMA pregnancies were excluded due to the small sample size, meaning that it would be unlikely that reliable conclusions could be drawn from this cohort, and specific MCMA complications, such as cord entanglement, which can lead to a high rate of fetal loss.

This left a total of 1567 pregnancies that were analysed in this study.



Maternal factors such as age, BMI at booking, ethnicity and parity were all assessed and grouped according to the chorionicity of the pregnancy. These characteristics are shown in table 2.

Maternal factors	DCDA (n=1193)	MCDA (n=374)	P-value
Maternal age at booking (median, IQR)	32 (28,36)	30 (26,34)	<0.001
Ethnicity (n, %)			
White (British, Irish, other)	1040 (87.2)	342 (91.2)	0.037
Black (African, other)	43 (3.6)	4 (1.1)	0.012
Asian	32 (2.7)	10 (2.7)	0.993
Chinese	8 (0.7)	5 (1.3)	0.215
Mixed	21 (1.8)	8 (2.1)	0.635
Other	34 (2.9)	4 (1.1)	0.051
Onset of labour (n, %)			
Spontaneous	297 (24.9)	89 (23.7)	0.667
Induced	363 (30.4)	106 (28.3)	0.447
Non-labouring C-section	533 (44.7)	178 (47.6)	0.323
BMI (kg/m^2) (Median, IQR))	25.3 (22.4, 29.7)	25.1 (22.3,28.9)	0.309
Previous mode of delivery (n, %)™			
None	533 (44.7)	194 (51.6)	0.014
Vaginal	526 (44.1)	151 (40.4)	0.196
C-section	129 (10.8)	29 (7.8)	0.086
Smoker status (n, %)			
Smoker	84 (7.1)	22 (5.9)	0.436
Non-smoker	464 (38.9)	169 (45.2)	0.03
Missing data	645 (54)	183 (48.9)	
Assisted reproduction (n, %)			
In vitro fertilisation	256 (21.5)	33 (8.8)	<0.001
Intracytoplasmic sperm injection	36(3.0)	7 (1.9)	0.237
Clomiphene	32 (2.7)	4 (1.1)	0.069

Artificial insemination	10 (0.8)	2 (0.5)	0.557
Intrauterine insemination	7 (0.6)	6 (1.6)	0.058
Gamete intrafallopian transfer	1 (0.1)	0	0.575
Human chorionic gonadotropin	1 (0.1)	0	0.575
None	494 (41.4)	203 (54.3)	<0.001
Missing data	356 (29.8)	119 (31.8)	
Parity (n, %)			
0	533 (44.8)	195 (52.1)	0.012
1+	660 (55.2)	179 (47.9)	0.012
Gestation at birth (weeks) (median, IQR)	37 (35, 37)	35 (32, 36)	<0.001
Birthweight (grams) (median, IQR)			
Twin 1	2480 (2110, 2790)	2160 (1640, 2460)	<0.001
Twin 2	2435 (2045, 2725)	2092.5 (1540, 2406.3)	<0.001
Average of twin set	2455 (2070, 2755)	2130 (1590, 2445)	<0.001
Birth weight discordance (median %, IQR)	9.9 (4.2,17.5)	10.6 (4.58, 19.73)	0.01
Birth weight centile (median, IQR)	25 (25, 25)	5 (5, 5)	<0.001
Neonatal death of fetus (n, %)	41 (1.7)	18 (2.4)	0.209
Fetal loss <24 weeks (n, %) per pregnancy			
Both fetus alive at 24 weeks	1167 (98)	357 (94.9)	0.002
Loss of one fetus <24 weeks*	15 (1.3)	13 (3.5)	0.005
Loss of both fetus <24 weeks*	9 (0.8)	6 (1.6)	0.141
Spontaneous fetal loss (of at least 1 fetus) <24 weeks	23 (1.93)	18 (4.81)	0.002
Iatrogenic fetal loss <24 weeks**	1 (0.08)	1 (0.27)	0.386
Number of stillbirths (n, %)	28 (1.2)	22 (2.9)	<0.001
Babies admitted to neonatal unit (n, %)	887 (37.2)	425 (56.5)	<0.001

*Intrauterine demise or miscarriage, **Pregnancy had undergone CVS/amniocentesis, ™missing data from 5 DCDA patients

Table 2 - Maternal, pregnancy and fetal variables separated by chorionicity

Over half of all mothers in both DC and MC pregnancies had a parity of 0 (44.8% and 52.1%) at booking (the twin pregnancy was their first pregnancy to pass 24 gestational weeks). There was a visible difference between the reported frequencies and this was shown to be statistically significant (p value=0.012).

The age of the mothers in the overall data ranged from 16 to 54 years. Median maternal age for MC and DC pregnancy was 30 and 32 years respectively. This difference was shown to be statistically significant with a P-value of <0.001.

BMI of the mothers at their booking appointment ranged from underweight at 16.7 kg/m2 to 53.6 kg/m2, with no significant difference in median value of 25.1 and 25.3 for MC and DC mothers (p-value 0.309). 52.4% of DC mothers were found to have an overweight or obese BMI of over 25 kg/m2 compared to 50% of MC mothers.

The ethnicity of the mothers was split into 6 different groups, with black mothers showing to make up a higher percentage of the cohort in DC compared to MC (3.6% vs 1.1%, black vs non-black p value 0.012). A statistical difference was also found with 91.2% MC and 87.2% DC of the pregnancies being born to white mothers (white vs non-white p value 0.037).

Spontaneous conception accounted for 54.3% of MC and 41.4% of DC pregnancies (p value <0.001). The most frequent artificial reproductive technique was In vitro fertilisation (IVF) which accounted for 21.5% of DC pregnancies contrasting only 8.8% of MC pregnancies. The difference in the use of IVF between the two chorionicities was found to be significant (p-value <0.001).

Gestation median was 35 weeks for MC and 37 weeks for DC pregnancies. The gestational weeks between the two chorionicitys were significantly different (p-value <0.001). The highest frequency for MC pregnancies were between 32-36+6 gestational weeks with 68% of all MC pregnancies being between these weeks. For DC pregnancies this was 37+ weeks with 52.2% of pregnancies in these weeks.

This cohort showed that spontaneous labour occurs in 24.9% of all DCDA pregnancies (297/1193) and 23.7% of all MCDA pregnancies (89/374). There was a similar percentage of induction (30.4% Vs 28.3%) and non-labouring c-section (44.7% Vs 47.6%) across both DC and MC pregnancies.

Median birth weight of twin 1 and twin 2 in a MC pregnancy was similar 2160g and 2092.5g. Median birth weight for twin 1 and twin 2 in a DC pregnancy was 2480g and 2435g. Birth weight discordance between MC and DC twins was also found to be significant (P value 0.01).

The data were separately split into smaller and larger twins for each chorionicities, and the distribution is shown in graph 4 and 5. In the MC cohort there was an over two-fold increase of still births compared to DC pregnancies (2.95% Vs 1.18%)(P value <0.001).

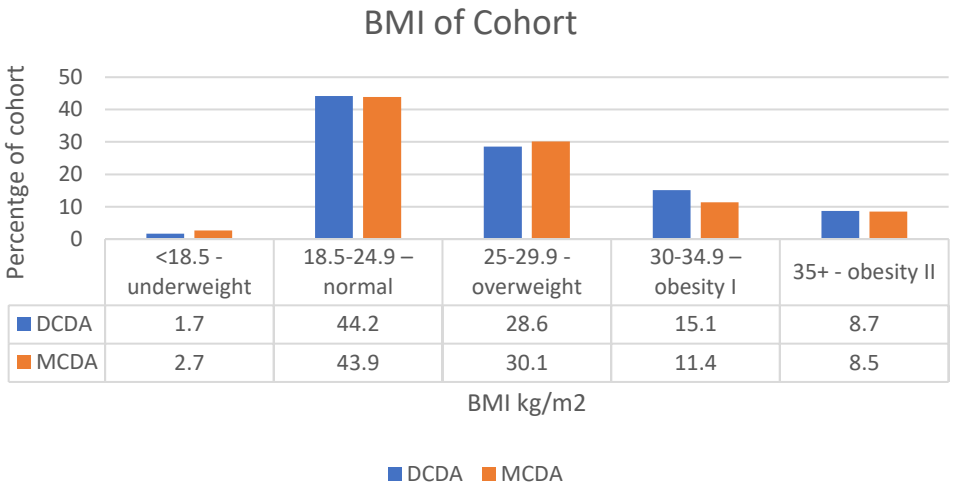


Figure 4 - BMI distribution of cohort split via chorionicity

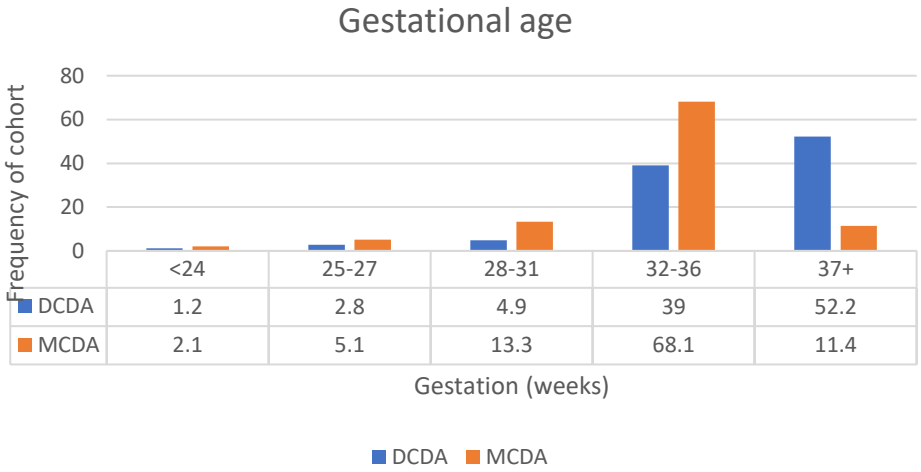


Figure 6 - Gestational age distribution of cohort split via chorionicity

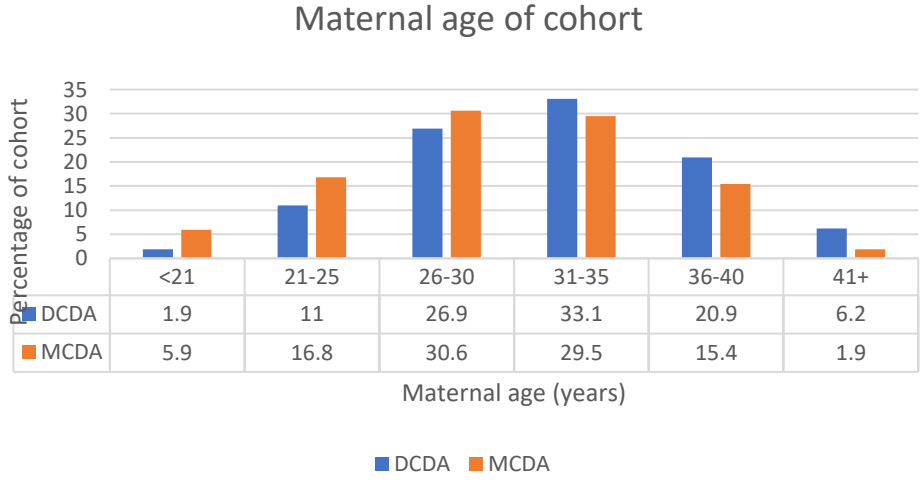


Figure 5 - Maternal distribution of cohort split via chorionicity

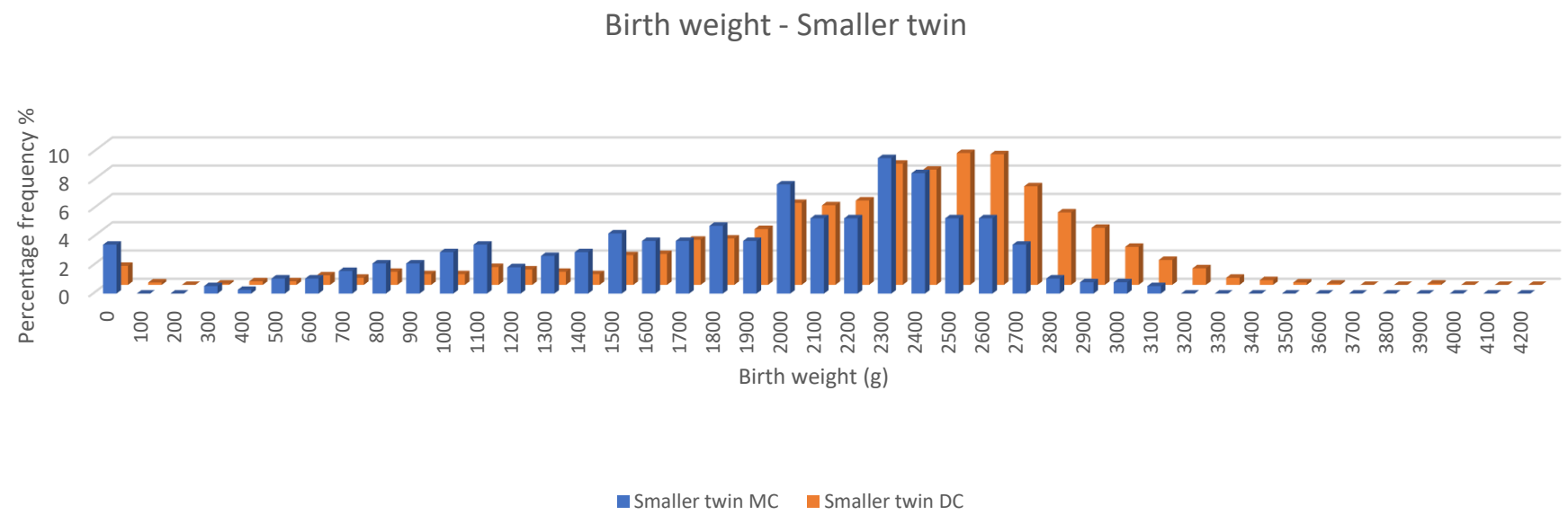


Figure 8 - Distribution of smaller twin birth weight for MC and DC fetuses

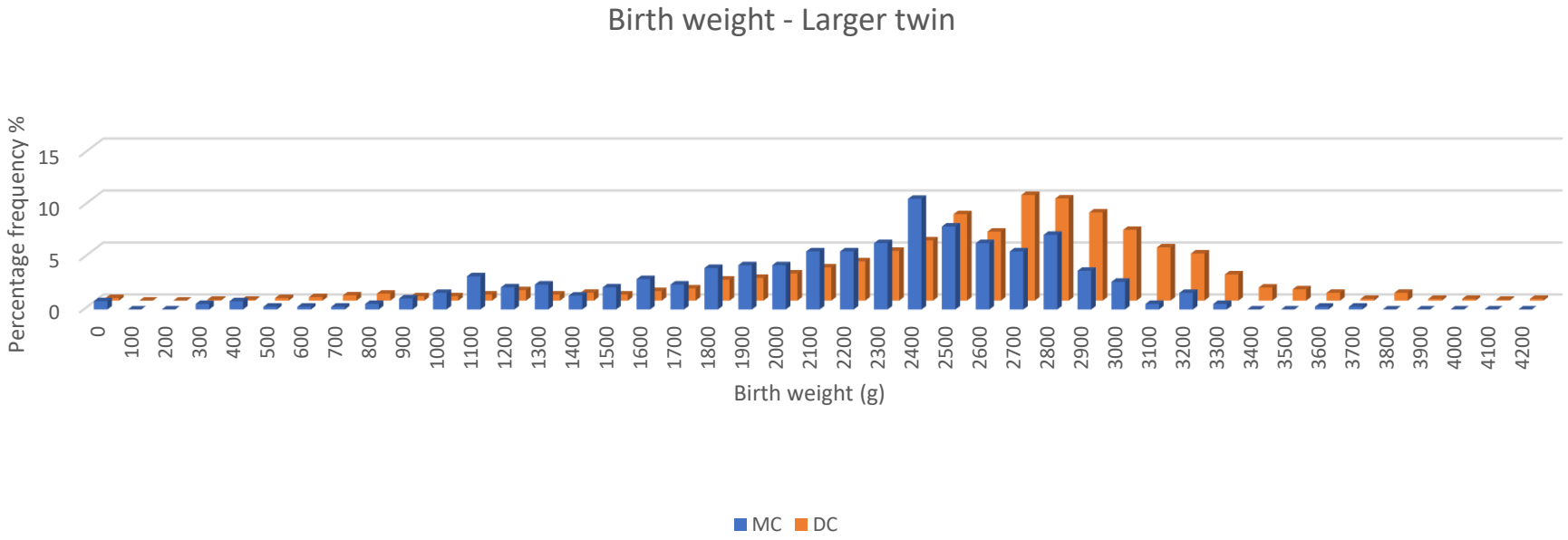


Figure 7 – Distribution of large twin birth weight for MC and DC fetuses

2.3.2 Fetal loss before 24 weeks gestation

Of the 1567 pregnancies identified, 43 of these were found to have a loss of one or both fetuses before 24 weeks, split into 24 DC and 19 MC pregnancies. Of these, a loss of one fetus occurred in 15 (1.3%) DC pregnancies and 13 (3.5%) MC pregnancies, with loss of both twins in 9 (0.8%) DCDA and 6 (1.6%) MCDA pregnancies.

	Total pregnancies with at least 1 fetal loss <24 weeks (n, %)	Risk of pregnancy having a fetal loss (per 100)	Relative risk	95% confidence interval
MC -1 loss	13 (3.5)	3.46	2.76	1.33,5.96
DC – 1 loss	15 (1.3)	1.26		
MC – 2 loss	6 (1.6)	1.6	2.13	0.76,5.94
DC – 2 loss	9 (0.8)	0.76		

Table 3 - The relative risk per pregnancy of losing one or both fetuses before 24 weeks in a MC pregnancy compared to a DC pregnancy.

	Total fetal losses <24 weeks (n, %)	Fetuses alive at 24 weeks	Risk of fetal loss (per 100)	Relative risk	95% confidence interval
MC	25 (3.34)	723	3.34	2.42	1.45. 4.04
DC	33 (1.38)	2353	1.38		

Table 4 - The relative risk of losing a fetus before 24 weeks in a MC pregnancy compared to a DC pregnancy.

Fetal loss <24 weeks		DCDA (n=24)	MCDA (n=19)	P-value
Mean Age at booking (years)		32.67 (SD-7.637)	29.05 (SD-6.041)	0.099
Ethnicity (n, %)				0.164
	White (British, Irish, other)	17 (70.8)	17 (89.5)	
	Black (African, other)	3 (12.5)	0	
	Asian	1 (4.2)	1 (5.3)	
	Chinese	0	0	
	Mixed	0	1 (5.3)	
	Other	3 (12.5)	0	
Onset of labour (n, %)				0.929
	Spontaneous	15 (62.5)	11 (57.9)	
	Induced	4 (16.7)	4 (21.1)	
	Non-labouring C-section	5 (20.8)	4 (21.1)	
BMI (kg/m^2) (average)		28.791 (SD-6.0946)	25.829 (SD-4.0989)	0.093
Previous MOD (n, %)				0.633
	None	12 (50.0)	12 (63.2)	
	Vaginal	8 (33.3)	6 (31.6)	
	C-section	3 (12.5)	1 (5.3)	
Smoker status (n, %)				0.949
	Smoker	1 (4.2)	1 (5.3)	
	Non-smoker	11 (45.8)	10 (52.6)	
Assisted reproduction (n, %)				0.903
	IVF	8 (33.3)	3 (15.8)	
	CLOM	2 (8.3)	1 (5.3)	
	None	9 (37.5)	5 (26.3)	
Parity (n, %)				0.625
	0	14 (58.3)	12 (63.2)	
	1+	10 (41.7)	7 (36.8)	

Table 5 - Pregnancies that lost at least one fetus <24 weeks gestation, split by chorionicity

Of these losses, 2 pregnancies were known to have undergone an invasive procedure, one loss occurred after amniocentesis (an MC pregnancy) and one after CVS (a DC pregnancy). Both pregnancies lost one fetus before 24 weeks and both lost the other fetus after 24 weeks gestation. The loss of the second fetus in the affected MC pregnancy occurred due to neonatal death at 27 weeks gestation and the affected DC pregnancy underwent a therapeutic termination at 30+3 weeks due to ventriculomegaly and small for gestational age (SGA). 7/19 (36.8%) of the MC pregnancies that had a loss of a least one fetus before 24 weeks had a diagnosis of TTTS.

Of the pregnancies that had 1 fetal loss before 24 weeks, 3/13 (23.1%) MC and 4/11 (26.7%) DC went on the lose the remaining twin after 24 weeks gestation, mostly through neonatal death due to preterm labour but with one therapeutic termination in a MC pregnancy.

2.4 Discussion

This study displayed that there are some significant differences in the maternal and pregnancy variables between MC and DC pregnancies.

This study reported that almost a quarter of the twin pregnancies were MC and with the remaining three quarters being DC. This was an expected result as DC twin pregnancies are more common due to all DZ fetuses being DC and 33% of MZ fetuses being DC (33). This leaves a smaller percentage being MC. This is comparable to other larger twin studies with a cohort of 20.1-28% MC and 72-79.9% DC pregnancies (47, 149). In contrast, a 2011 Swedish study found a lower MC twinning rate of 13.8% (150). However, this study suggested the difference in this number may be due to different rates of artificial reproductive techniques resulting in more DC pregnancies. This current study was a large retrospective cohort study of 1567 pregnancies which could suggest the distribution of chorionicity is similar to that seen in the general population.

There were a large range of ART techniques in this cohort but only IVF was found to have a significant difference between the MC and DC twins (8.8% vs 21.5% p value <0.001). A known complication of IVF is a twin pregnancy, with DC twins being more common due to these twins resulting from either two embryos or one embryo splitting after fertilisation. The NICE guidance states that no more than two embryos should be transferred during one IVF cycle (151), however this can still pose the risk of a DC pregnancy if both embryos develop successfully. Spontaneous fertilisation was also found in this cohort to be significantly different between the two groups (p

value <0.001), however there was a large amount of data missing from this variable (approximately 30% for both chorionicities), through it not being reported on the women's pregnancy history. Despite this, the percentage of missing data for each chorionicity was similar, resulting in this unlikely to have affected the results.

A statistical difference was found between the chorionicity of pregnancies in black vs non-black women, with a higher percentage seen in DC pregnancies (1.1% MC vs 3.6 DC);(p value 0.012). In previous literature, black women have been reported to have higher rates of DC twin births than in non-back women, with the most recent data from the USA reporting a twin birth rate of 42.0 per 1,000 births in black women compared to 35.4 and 25.1 in white and Hispanic women (152).

Median maternal age showed a significant difference between the two pregnancy cohorts (p value <0.001). This result was expected as a DC pregnancy is more frequently seen in women of an increased age compared to an MC pregnancy. Previous studies showed similar results with mean maternal age being significantly ($p<0.05$) lower in MC pregnancies compared to DC pregnancies (2, 47). This is most likely due to a higher percentage of DC pregnancies being born through IVF, where there is a longer time frame of trying to spontaneously conceive and then going through the IVF process.

This study also found that MC twins had both a significantly lower gestational age at delivery and median birth weight than DC twins. However, the median birth weight was not adjusted for by gestation so further analysis would be needed to see if this weight discrepancy persists after adjusting for gestational weeks. Despite this, median birth weight centile was shown to be significantly different between the two chorionicities, suggesting that the significant result seen for median birth weight would still be significant after adjustment for gestational age. Birth weight discordance was also shown to be significantly different between MC and DC pregnancies. This can likely be explained by cases of TTTS in the MC pregnancies resulting in a higher birth weight discordance between the twin pair.

Similar findings were found in a 2013 study on twins (2) with the mean birth weight found to differ by 285g between chorionicities (p value <0.01). MC pregnancies have found to be overall, higher risk pregnancies than DC pregnancies with complications such as TTTS, sFGR and fetal and neonatal deaths all found to be increased. This could lead to higher levels of obstetric intervention; despite this, similar rates of induction and non-labouring C-section were seen in this cohort study across MC

and DC twins. However, this birth intervention could be at an earlier gestation dependant on the complication of the pregnancy.

In this cohort, 47 MC (12.5%) pregnancies were found to have a diagnosis of TTTS at some point during the pregnancy. Other studies have found a rate varying from 5-15% (153), meaning the findings of this cohort study are in the range of previous literature values.

The stillbirth data from this cohort showed a significant ($p<0.001$) difference between MC and DC pregnancies with the stillbirth rate in the MC group being over double the rate of the DC group (2.9% Vs 1.2%). Previous data has shown that MC twins have the highest rate of stillbirths and fetal loss supporting this study's findings (154-157). 10 of the stillbirths in this cohort were in pregnancies that had been diagnosed with TTTS, if these pregnancies were excluded, this results in a 1.6% (12/738) stillbirth rate in the MC twins, a figure still increased but much closer to the stillbirth rate seen in the DC twins. This suggests that the vascular complications seen in MC twins play a large part in the higher rate of stillbirths, although a MC chorionicity still seems to be at higher risk of stillbirth even without the added complication of TTTS. This finding has also been observed in other studies showing that MC twins have higher rates of inter-utero complications despite excluding unique complications of a MC placenta (156, 158).

Overall, this study has demonstrated that a MC pregnancy has a higher risk of losing at least one fetus before 24 weeks than a DC pregnancy, 25/748 (3.34%) vs 33/2386 (1.38%) fetuses lost with a relative risk of 2.42 (CI 1.45, 4.04).

The data also show that women with MC pregnancies are at higher risk of miscarrying one fetus (relative risk 2.76 CI 1.33,5.96) and both fetuses (relative risk 2.13 CI 0.76,5.94) when compared to a DC pregnancy although this difference was only statically significant for loss of one twin <24 weeks (p value 0.005). This was an expected result and is in line with previous studies on miscarriage rate in twins (159, 160). MC pregnancies are higher risk due to complications such as TTTS, and studies have shown a 25% risk of subsequent death in the remaining co-twin if one twin has died. This same study did not find any similar link in DC pregnancies (161).

One study found that the median gestational age for the death of one twin is comparable in MC and DC pregnancies but the median gestational age for the co-twin was lower in a MC pregnancy ($p<0.002$)(162). Other studies have reported a difference in the co-twin survival rate with it being

markedly increased in DC (71%) compared to MC (32%) (163). This result suggests that the survival of each twin is more closely linked in a MC pregnancy compared to a DC pregnancy, largely due to the nature of the cause of fetal demise with vascular connections within the placenta predominate in MC pregnancies. This retrospective cohort study only collected data on death before 24 weeks and not specific gestation so is unable to report such findings. In this study, the rate of loss in the co-twin after 24 weeks was found to be similar (26.7% DC and 23.1% MC), with all but one loss due to neonatal death by complications of prematurity. The remaining twin was lost due to a post 24 weeks therapeutic termination.

Spontaneous miscarriage rates in this cohort study were 1.93% for DC and 4.81% in MC pregnancies. Previous studies have shown spontaneous miscarriage rate to be similar for DC but slightly higher than this study's findings for MC with 9.5% MC and 1.4% DC (47). The difference in the MC miscarriage rate could be explained as this study examined twin cases delivering in 2010-2020, reflecting modern surveillance and protocol driven management. MC pregnancies can be complicated by vascular events and this data could be influenced by modern interventions and technologies that have led to identification and treatment of these complications, leading to a lower rate of spontaneous miscarriage. Definitive treatment of TTTS, seen in MC twins, is a fetoscopic laser photocoagulation, which effectively creates two separate chorions, each supplying their own twin (164).

In 2003, a study looking at IVF/ICSI conceived twins pregnancies reported a total risk of spontaneous fetal loss of 11.1% (P value <0.01)(165). In this retrospective cohort study, it was found that 33.3% of the DC pregnancies that lost at least 1 fetus before 24 weeks were conceived via IVF. It was also found that 15.8% of MC pregnancies that had a loss of one or both fetus were conceived via IVF (background 8.8%). Whilst these are higher frequencies in the miscarriage group as opposed to the overall cohort, the cohort size of miscarriage rate was small at 43 pregnancies and this could be the result of a type 1 error. For this result to be significant, further studies would have to be done specifically into IVF and twin miscarriage rates.

Other studies have found total fetal losses before 24 weeks to be 8.2-14.2%% for MC and 2.6% for DC (P<0.01)(150) with an OR=6.1(47). This is in line with this study's finding of MC twins at a higher risk of miscarrying compared to DC twins 3.32% versus 1.39% (OR 2.45), but this cohort study shows a lower rate than other reported risk levels.

An analysis of the Southwest Thames Obstetric Research Collaborative multiple pregnancy cohort found a significantly higher risk of fetal loss in MC twins (60.3 per 1000 foetuses) than in DC twins (6.6 per 1000 foetuses) with a relative risk of 9.18 (95% cl, 6.0-13.9) (166) This retrospective cohort study has found a higher miscarriage rate in MC pregnancies compared to DC pregnancies with a relative risk of 2.37 (95% CI- 1.44-4.01). Although the relative risk between the two data sets is quite different, both studies show an increased miscarriage risk in MC twins compared to DC twins. As previously mentioned, this data set is from the previous 11 years (2010-2020), with the STORK cohort being a 10-year study from 2000. The reduction in relative risk between the two studies could be explained by advances in technology and treatment of MC pregnancy complications.

No significant differences were found in the variables between MC and DC twins that had lost one or two fetuses before 24 weeks (Table 5) despite significant differences found between the chorionicities in the total population (Table 2). This finding can most likely be explained through the small sample size in this group leading to many of the comparisons being underpowered. Due to this, no conclusions can be drawn from table 5.

2.5 Strength and limitations

A strength of this study is that this is a large cohort of both DC and MC twins. Aside from smoking status and artificial reproductive techniques, only a small amount of data was missing for each variable. These women had their care in the same hospital and were monitored in the same way. These women will have had consistent management and observation throughout pregnancy. It also means that gestation and chorionicity were determined using the same methods so should be consistent across the whole cohort.

A limitation of this study was that no maternal health data was collected. Maternal health may have played a part in the miscarriage rate and gestation of pregnancies, and this could make this dataset more robust at predicting the real risk of miscarriage in a twin pregnancy. Another limitation is that that zygosity of the pregnancies wasn't determined although in reality this is very difficult to do and not routine practice. However other twin studies have shown that it is chorionicity rather than zygosity that is linked to fetal outcomes and pregnancy outcomes (167) and therefore out cohort reflects clinical management.

2.6 Conclusion

This study has demonstrated that MC twin pregnancies have an increased risk of fetal loss before 24 weeks when compared to a DC twin pregnancy. However, the relative risk and percentage frequency of fetal loss before 24 weeks is reported as lower than seen in other twin cohort studies. This could be explained by this cohort being from a more recent time period, the last 11 years (2010-2020), in comparison to previous older studies with data from the early 2000s. This study also found a higher frequency percentage of IVF conceived pregnancies affected by <24-week fetal loss than seen in the overall cohort suggesting these pregnancies maybe at higher overall risk of early fetal loss. Further work would be required to examine this signal.

Chapter 3 : Predictors of a preterm birth in a twin pregnancy

3.1 Introduction

Preterm birth (PTB) is defined by the world health organisation (WHO) as a birth before 37 completed weeks gestation (103, 145), or prior to 259 days from the first day of the pregnant woman's last menstrual period before the pregnancy (168). Rates of PTB within the UK are reported at 7.3% of live births (145). However this varies by location and with number of fetuses carried, rates in low risk singleton pregnancies range from 3.6-14.7% of live PTBs (169). It has been found that nearly 85% of PTB occur in the late pre-term stage (34-36+6 weeks), and globally, in 2014, 80% of these were born in Asia and sub-Saharan Africa (170).

PTB is the most significant cause of morbidity and mortality amongst neonates. It is estimated that a neonate born at 24 weeks has a 44% risk of mortality and 71.4% have a major neonatal morbidity (171). At 25 weeks, 45% of the survivors go on to have moderate to severe handicaps throughout child and adulthood (5). PTB, and its associated complications, has found to be the leading cause of death in children <5 years old (172). Common neonatal complications include respiratory distress syndrome, sepsis, necrotising enterocolitis and intraventricular haemorrhage, all of which inversely correlate to the gestational age at delivery and contribute to short and long term complications.
It can be useful to divide PTB into spontaneous or iatrogenic categories, although both can result from the same complication (such as maternal ill health). Approximately, 3/4 of PTB's are spontaneous, with the remaining 1/4 due to healthcare intervention (145).

Twin pregnancies are classed as high risk and are associated with an increase in maternal morbidity; including hypertensive disorders, anaemia, gestational diabetes and venous thromboembolism, and increased maternal mortality (173). A study in 2012 found that 34% of monochorionic (MC) pregnancies are classed as complicated by the time they reach 34 weeks gestation, with 29% of dichorionic (DC) pregnancies being classed as complicated by the time they reach 36 weeks (174). Twin pregnancies have higher levels of PTB contributing 15-20% of all preterm births despite only making up 2-3% of all births (4). A meta-analysis found a rate of twin births before 32, 34, and 37 weeks to be 7%, 13% and 41% (175).

Given the higher risks of preterm birth in the twin population, the prediction and prevention of such births is a clear objective to investigate and achieve. It is also important to separate outcomes according to chorionicity to see if they need to be treated as two separate populations or as one.

3.1.1 Routine Management of twin pregnancy

At LWH, twin pregnancies are cared for within the multiple pregnancy clinic (MPC). Once the twin pregnancy is discovered at the first trimester dating scan, the women should be referred to the multiple pregnancy clinic at this point and be seen within 3 weeks at 16 weeks gestation.

The dating scan is important as it is at this point that chorionicity, amnionicity and viability can be best determined; chorionicity should be determined <14 weeks (66). If either chorionicity or amnionicity cannot be determined during the dating scan, the woman should be referred to the MPC to have a consultant scan within 2 weeks. Until chorionicity can be determined, the pregnancy should be treated as MC due to the increased risk of complications and the need to optimise antenatal management. If transabdominal ultrasound has not been successful in determining chorionicity (common causes are high BMI or retroverted uterus) then transvaginal assessment is suggested.

During the dating scan, nomenclature should be given to the babies, this is the position of the babies such as upper and lower or left and right. This should be clearly documented in the mothers notes to ensure the twins can be identified in each subsequent scan.

If MC twins are identified and a fetal heartbeat cannot be found in one fetus, the pregnancy should be referred to fetal medicine to ensure that twin reversed arterial perfusion (TRAP) can be excluded. At the dating scan, women should also be offered screening for trisomy 21, 18 and 13 (Downs syndrome, Edwards syndrome and Patau syndrome). In twins this involves the combined nuchal translucency test (nuchal measurement and first trimester serum screening test). This should be ideally be performed between 11-12+6 gestational weeks, with the latest gestation being 14+1 weeks or crown rump length of 84mm (176). If the pregnancy is past this gestation, the women should be offered second trimester quadruple screening for Down syndrome, which can be done up to 20+0 weeks(176). In an MC pregnancy, the chance of trisomy 21 is lower than a singleton (due to high fetal loss), but T21 rates are higher in a DC pregnancy when compared to a singleton pregnancy (detection rates 80% compared to 40-50%) (176). The mother should be informed of the different

detection rates in her specific pregnancy, and in a DC pregnancy, informed that the test is not fetal specific (may be unable to tell if just one baby is affected or both).

The frequency and type of scan offered to twin mothers are dependent on chorionicity.

3.1.2 DC twins

DC twins should have growth scans at 24, 28, 32 and 36 weeks' gestation in line with national institute for health and care excellence (NICE). Each scan should report on both twins estimated fetal weight with concerns about fetal growth, liquor volume or the wellbeing of one or both twins during the scans, necessitating referral to the MPC. Additional midwifery appointments without scans should be offered at 16 and 34 weeks' gestation.

3.1.3 MC twins

MC twins should be scanned every fortnight from 16 weeks gestation until delivery by a consultant or under the supervision of a consultant in the MPC. Each scan should report on the estimated fetal weight of each twin, biometry (head circumference, abdominal circumference and femur length), liquor volume, fetal dopplers including umbilical artery, middle cerebral artery and ductus venosus and bladder size. If there is any discrepancy in fetal size, liquor volume or dopplers the scan findings should be reviewed by the MPC lead under suspicion of twin-to-twin transfusion syndrome (TTTS), selective fetal growth restriction (sFGR) or twin anaemia polycythaemia sequence (TAPS). The frequency of monitoring should be increased to at least weekly if there are any concerns about discordant amniotic fluid levels.

If a pregnancy is found to be monochorionic monoamniotic (MCMA), they should have additional scans and monitoring. These include scans every fortnight from 16 weeks gestation until delivery. Each scan should report on liquor volume, size or fetal bladders, estimated fetal weight and assessment of the umbilical artery, middle cerebral artery and ductus venosus of each twin with a colour flow doppler. If there is any suspicion or evidence of cord entanglement, weekly scans should be considered (66, 177). Cord entanglement is a frequent finding in MCMA twin pregnancies but the commonest cause of fetal loss is fetal abnormalities with studies reporting rates of congenital abnormalities (in at least one twin) as high as 27% (178, 179).

Other care offered during the pregnancy is a full blood count at 20-24 weeks gestation due to the higher risk of maternal anaemia, and appropriate supplementation should be offered at this point if needed (haemoglobin <10.6 g/dl). It is not recommended for a twin pregnancy mother to have an assessment of the cervix unless clinically indicated, in contrast to higher order multiple pregnancies, (66, 177). This recommendation was due to inconsistencies between the studies, but also a lack of an effective intervention for preterm birth (66). However, LWH routinely screens women at 16 weeks with a cervical length scan. The ISUOG guidance states that cervical length can be measured in the second trimester and a cut off of 25mm is normally used as a screening risk factor for preterm birth (180)

3.1.4 Birth care

The birth plan including timing and method of delivery should be discussed at 28 weeks' gestation or earlier. The risks and benefits of the different modes of delivery should be discussed with the mother. Analgesia during labour, intrapartum fetal monitoring and management of the third stage of labour should be discussed.

At LWH, induction of birth is offered to mothers from 37 weeks in DC and from 36 weeks in MC pregnancies as per NICE guidance (66). The method of delivery depends on the presentation of the leading twin.

In an uncomplicated MCDA or DCDA pregnancy with a cephalic (head down) leading twin, the mother is offered to an induction of labour and or caesarean section. Both these modes of labour can be offered as long as the pregnancy is uncomplicated, past 32 weeks, no obstetric contraindication to labour and there is no significant size discordance between the twins (66).
If the mother does not want to be induced at 37 weeks, she should be informed of the risks of continuing a twin pregnancy past this stage, and reviewed by the MPC if she reaches 39 weeks' gestation.

In any complicated pregnancy such as a MC twin pregnancy with a diagnosis of TTTS, TAPS or death of one twin, birth timing and mode of delivery should be determined on an individual case by case basis by the MPC medical team. The risk of a caesarean section and the potential impact of perinatal mortality and neonatal morbidity should be weighed up.

A MCMA pregnancy should be delivered by caesarean section between 32+0 and 33+6 weeks or after any complication is diagnosed requiring earlier delivery (66, 177). The earlier delivery is due the risk of cord entanglement seen in MCMA due to the lack of a separating membrane between the fetuses.

3.2 Method

3.2.1 Data collection

Data was collected from the LWH Meditech online software where all booking and delivery data is stored. Chorionicity was initially determined from discordance sex twins (clearly recorded as male and female) and classified as DC twins. Viewpoint was then searched to determine chorionicity of male-male and female-female twin pairs, which was determined from the dating scan. Chorionicity was occasionally reported on the birth summary, this was used to cross reference the information found on viewpoint.

Any higher order multiple pregnancies (triplets or above) were excluded, including any pregnancy that had been reduced to a twin pregnancy through fetal loss or selective reduction/fetocide. This is due to the increased risk in a higher order multiple pregnancy and the risk on the remaining live fetuses after fetal death or selective reduction.

Data was not collected on any twin pregnancy that had their initial antenatal care at another trust and then subsequently delivered at LWH. This is due to LWH being a tertiary centre receiving a large amount of complicated pregnancy referrals. By including these referred pregnancies, this may have inflated the PTB rate seen at LWH and therefore increased the PTB rate seen in this study. Gestation of the twin pregnancy was determined from the dating scan and was reported both on pregnancy history and delivery summary of the twin pregnancy.

Maternal BMI, ethnicity, smoker status, artificial reproductive techniques, parity, age, and previous cervical surgery was determined from the initial booking appointment. Further information on cervical surgery was obtained from cytology and histology results on ICE and scanned clinic letters. Cervical surgery was determined from a history of a loop excision or cone biopsy procedure.

Any previous PTB was determined from pregnancy history, with the details including gestation and onset of labour taken from previous birth summaries. Previous history of a PTB is defined as any delivery <37-week recorded on the mother's pregnancy history. Previous method of delivery in a multiparous woman was recorded only from the immediately preceding delivery. Smoking was defined as any mother actively smoking at least one cigarette during the pregnancy period. Any history of past smoking was not accounted for. Parity was determined from any previous pregnancy that had surpassed 24 weeks gestation.

This study classified a twin PTB if it occurred <34 weeks. This decision was taken as these earlier gestations present the greatest clinical challenge and have the highest morbidity/mortality (181, 182) and in addition iatrogenic births are more common in the late PTB cohort (34-36+6 weeks).

3.2.2 Statistical analysis

Analysis was performed on the impact of clinical and demographic factors on the gestational age at delivery in weeks. The primary outcome was a preterm birth which is classed as <34 weeks. Continuous data was summarised as median (inter-quartile ranges), and categorical data summarised as frequencies (percentages). Analysis was performed using generalised linear models technique assuming normal and binomial distribution and applying identity and logistic link function respectively.

Univariable and multivariable analysis was performed to assess the impact of clinical/demographic covariates on the study outcome. Multivariable analysis was done on all candidate covariates using a backwards step-wise process based on Akaikes information criterion (AIC).
Statistical significance was set at a p value of 0.05. The univariable and multivariable analysis was completed by Dr Richard Jackson.

3.3 Results

1584 twin pregnancies (3168 babies) born between January 2010 and November 2020 at LWH were included in this study. Of the twin pregnancies collected, 1193 of these were dichorionic (DC) and 391 were monochorionic (MC). The collected data was separated into two groups, delivery before 34+0 weeks gestation and delivery at 34+0 or more weeks gestation. Of the twin pregnancies

analysed, 20.8% (329/1584) were born before 34 weeks and 79.2% (1255/1584) were born at or after 34 weeks.

3.3.1 Univariable analysis

Table 1 details the summary statistics and univariable analysis of the 1584 patients. Those termed significant at a 5% level are highlighted. This analysis is split separately into gestation time (continuous value) and <34 weeks and ≥ 34 weeks (categorical value).

				Gestation Time		Gestation ≥ 34 weeks		
Variable	<34+0 weeks	≥34+0 weeks	Total	Effect on gestation (weeks) (se)	Pval	Effect on gestation (weeks) (se)	OR (95% CI)	Pval
Total (n)	329	1255	1584					
Ethnicity								
Asian	13 (4.0%)	41 (3.3%)	54					
Black	6 (1.8%)	39 (3.1%)	45	-0.24 (0.67)	0.719	0.64 (0.549)	1.9 (0.648, 5.572)	0.242
Mixed/Other	18 (5.4%)	80 (6.4%)	98	-0.09 (0.566)	0.876	0.31 (0.424)	1.36 (0.591, 3.122)	0.47
White	292 (88.8%)	1095 (87.2%)	1387	-0.16 (0.462)	0.736	0.14 (0.336)	1.14 (0.593, 2.211)	0.687
Artificial reproduction								
Clomifene	8 (2.4%)	28 (2.3%)	36					
ICSI	7 (2.1%)	36 (2.9%)	43	1.16 (0.738)	0.118	0.38 (0.576)	1.47 (0.476, 4.541)	0.504
IVF	60 (18.2%)	230 (18.3%)	290	0.54 (0.577)	0.354	0.09 (0.426)	1.1 (0.475, 2.526)	0.831
NONE	245 (74.5%)	943 (75.1%)	1188	0.76 (0.553)	0.171	0.12 (0.408)	1.13 (0.507, 2.505)	0.769
Other	9 (2.7%)	18 (1.4%)	27	-0.3 (0.832)	0.722	-0.56 (0.572)	0.57 (0.186, 1.754)	0.328
Parity								
0	185 (56.2%)	554 (44.1%)	739					

1	88 (26.7%)	409 (32.6%)	497	0.78 (0.19)	<0.001	0.4 (0.147)	1.49 (1.116, 1.983)	0.007
2+	56 (17.1%)	289 (23.0%)	345	0.88 (0.214)	<0.001	0.5 (0.171)	1.65 (1.182, 2.31)	0.003
Previous PTB								
No	291 (88.4%)	1189 (94.7%)	1480					
Yes	38 (11.6%)	66 (5.3%)	104	-1.43 (0.339)	<0.001	-0.93 (0.219)	0.39 (0.257, 0.606)	<0.001
Prev cervical surgery								
No	306 (93%)	1175 (93.6%)	1481					
Yes	23 (7.0%)	80 (6.4%)	103	-0.15 (0.335)	0.651	-0.07 (0.249)	0.93 (0.572, 1.52)	0.778
Maternal age (median, IQR)	31 (27, 35)	32 (28, 35)	32 (27, 35)	0.03 (0.015)	0.05	0.02 (0.011)	1.02 (0.994, 1.038)	0.158
Smoker								
No	127 (38.6%)	514 (41.0%)	641					
Yes	24 (7.4%)	82 (6.6%)	106	-0.1 (0.351)	0.772	-0.08 (0.265)	0.92 (0.549, 1.554)	0.765
Missing data	178 (54%)	658 (52.4%)	836	-0.11 (0.174)	0.509	-0.1 (0.132)	0.9 (0.697, 1.171)	0.443
BMI (Median, IQR)	25 (22.1, 29.2)	25.3 (22.4, 29.6)	25.2 (22.4, 29.5)	-0.02 (0.014)	0.125	0 (0.01)	1 (0.979, 1.019)	0.888
Chorionicity								
DC	192 (58.4%)	1001 (79.8%)	1193					
MC	137 (41.6%)	254 (20.2%)	391	-1.82 (0.188)	<0.001	-1 (0.135)	0.37 (0.284, 0.481)	<0.001

Table 6 – Univariable analysis of variables split into continuous (length of gestation) and categorical (<34 weeks or ≥34 weeks) analysis for all twin pregnancies

Univariable analysis was undertaken of the data and the results are shown via the variables effect on gestational time of the twin pregnancy.

3.3.1a Monochorionic

When splitting the data by gestation, it was found that 16% and 35% of the DC and MC twin pregnancies respectively were delivered before 34 weeks. This was found to be a significant difference between the two chorionicities with MC pregnancies reducing the gestation by 12.7 days when compared to a DC pregnancy (P value <0.001). When comparing the data by <34 weeks and ≥34 weeks groups, MC pregnancies were found to have a shorter overall gestation time (OR 0.37).

3.3.1b Parity

Being a multiparous mother (having had a previous pregnancy past 24 weeks) appears to be a protective factor against PTB in the subsequent twin pregnancy. Both having a parity of 1 and 2+ prolongs pregnancy by 5.46 and 6.16 days in the twin pregnancy (significant finding with both P values <0.001).

3.3.1c Previous preterm birth

6.57% (104/1584) of the cohort had previously experienced a <37-week PTB. In the <34 week group this rate was shown to be 11.6% (38/329) versus 5.3% in the >34-week group. Having a previous PTB had a detrimental effect on the gestation of the subsequent twin pregnancy with a reduction of 10 days gestation (significant P value of <0.001). Of the women who had had a previous PTB, 37% then had a subsequent preterm twin pregnancy, with 63% going on to deliver the twin pregnancy at 34+0 weeks or more. This is in comparison to the women without a previous history of PTB, only 20% of these women delivered preterm with 80% delivering at 34 weeks onwards.

3.3.1d Maternal age

Maternal age was also shown to have a small but significant effect on gestation. For every yearly increase in maternal age (from 16 years, taken from booking appointment), there is a 0.21 day increase in the gestation of the twin pregnancy (P value 0.05). This is only found when looking at the data at gestational time, not when splitting the data into preterm (<34 weeks) and term (≥34 weeks) groups.

3.3.2 Multivariable analysis

Multivariable analysis was performed for both a continuous value (gestational time) and a categorical value (gestation ≥34 weeks) separately.

3.3.2a Gestational time

For gestational time, table 7 shows the significant variables that had an impact on gestation.

	Effect on gestation (weeks) (se)	**Pval**
(Intercept)	35.92 (0.362)	<0.001
Parity 1	0.88 (0.184)	<0.001
Parity 2+	1.14 (0.216)	<0.001
Previous PTB	-2.08 (0.338)	<0.001
BMI	-0.03 (0.013)	0.014
Chronicity MC	-1.79 (0.185)	<0.001

Table 7 - Multivariable analysis for length of gestation

As with the univariable analysis, this shows that a parity >0, previous PTB and a chorionicity of MC had a significant impact on gestation. The multivariate analysis has shown that a parity of 2 increases the gestation by 7.98 day, higher than what was seen in the univariable analysis.

After multivariable analysis, booking maternal BMI was found to decrease the twin pregnancy gestation by 0.21 days for every 1kg/m2 increase in BMI (P value 0.014). This finding was seen in univariable analysis with similar impact on gestational time but found to not be statistically significant.

	DC		MC	
	Effect on gestation (weeks) (se)	**Pval**	**Effect on gestation (weeks) (se)**	**Pval**
(Intercept)	35.26 (0.418)	<0.001	35.41 (0.369)	<0.001
Parity 1	1.05 (0.203)	<0.001	0.94 (0.19)	<0.001
Parity 2	1.24 (0.233)	<0.001	1.29 (0.222)	<0.001
BMI	-0.01 (0.015)	0.533	-0.03 (0.013)	0.019
Previous PTB	-2.36 (0.357)	<0.001	-2.00 (0.348)	<0.001

Table 8 - Multivariable analysis for length of gestation by Chronicity Type

Multivariable models for DC and MC groups separately are included in Table 8

The variate affects across both chorionicities are almost the same, each with similar effects on gestational time. Having a previous PTB has a greater impact on a DC pregnancy, with this reducing the gestation by 16.5 days compared to a reduction of 14 days in a MC pregnancy (P value <0.001).

Of interest may be that the impact of BMI is more pronounced on MC patients as opposed to DC, with only the effect on a MC pregnancy being significant (P value 0.019). This displays a reduction in gestation time of 0.03 weeks for every 1kg/m2 increase of booking maternal BMI.

An adjusted R2 value for model fits of 0.07 is obtained suggesting just 7% of the residual variance is explained by the model highlighting a large amount of noise in the data poor predictive ability.

3.3.2b Gestation ≥34 weeks

Multivariant analysis was conducted separately into gestation <34 and ≥34 weeks. Table 9 shows these results

	Effect on gestation (se)	**OR (95% CI)**	**Pval**
(Intercept)	1 (0.091)	2.71 (2.271, 3.244)	<0.001

Parity 1	0.52 (0.137)	1.69 (1.289, 2.205)	<0.001
Parity 2+	0.62 (0.164)	1.85 (1.344, 2.556)	<0.001
Prev PTB	-1.34 (0.229)	0.26 (0.167, 0.411)	<0.001
Chorionicity MC	-1.06 (0.127)	0.35 (0.27, 0.443)	<0.001

Table 9 - Results of Multivariable model for gestation >34 weeks

In this analysis, a parity of >0 shows a prolongation of the twin pregnancy gestation, with a parity of 1 increasing by 3.64 days (OR 1.69) and parity of 2 increasing by 4.34 days (OR 1.85). Both a previous preterm birth and having MC twins shows a reduction in gestation weeks. All these variables are shown to be significant with a P value <0.001. Unlike with gestational time analysis, analysing by preterm and term pregnancies, BMI was not found to have a significant impact on gestational weeks.

An AUC model of 0.66 shows that these predictive variable for preterm birth can be used to accurately predict a ≥34-week twin birth in 66% of subsequent twin pregnancies.

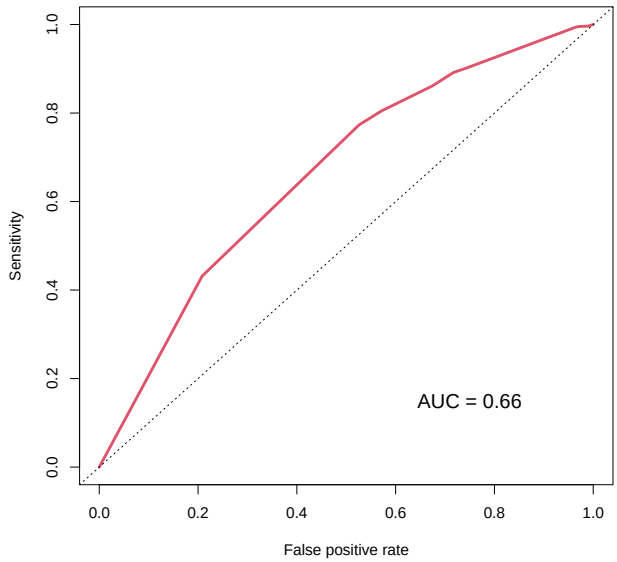


Figure 9 - AUC for the fit of model explaining gestation >34 weeks

This multivariable analysis was further split by chorionicity into DC and MC pregnancies.

This data shows comparable covariate effects between the two patient groups, with each variable have a slightly more impact on the gestational time in the DC cohort.

	DC			MC		
	Effect on gestation (weeks) (se)	**OR (95% CI)**	**Pval**	**Effect on gestation (weeks) (se)**	**OR (95% CI)**	**Pval**
(Intercept)	1.37 (0.108)	3.95 (3.2, 4.882)	<0.001	1.1 (0.085)	3.02 (2.553, 3.563)	<0.001
Parity 1	0.74 (0.196)	2.1 (1.431, 3.08)	<0.001	0.56 (0.149)	1.75 (1.306, 2.339)	<0.001
Parity 2+	1.00 (0.239)	2.71 (1.693, 4.324)	<0.001	0.87 (0.188)	2.39 (1.653, 3.45)	<0.001
Previous PTB	-1.56 (0.278)	0.21 (0.121, 0.361)	<0.001	-1.33 (0.236)	0.27 (0.167, 0.422)	<0.001

Table 10 - Multivariable analysis for Gestation ≥ 34 weeks by Chorionicity Type

3.4 Discussion

3.4.1 Principal findings

This study has confirmed that multiparity is a protective factor in women embarking on a twin pregnancy, with gestation prolonged in these pregnancies. A previous PTB and sharing of a placenta decrease the gestation at delivery of a twin pregnancy. Both the univariate and multivariate analysis showed a previous term birth is a protective factor against preterm twin birth and that having a parity of 2+ prolonged the twin pregnancy further than a parity of 1. A previous PTB was the most significant factor for decreasing the gestation of the twin pregnancy, and therefore a potential predictor of preterm birth.

3.4.2 Existing literature

3.4.2a Previous preterm birth

A previous preterm birth has been shown in existing literature to be a predictor of subsequent preterm birth in a twin pregnancy. A 2014 systematic review found that 11.9% of preterm twins were born to mothers who had previously had a preterm singleton pregnancy (183). This current

cohort study found an almost identical rate of 11.6%, however this study classed a twin preterm birth as <34 weeks, with the systematic review classing <37 weeks as a preterm birth. This concludes that this current cohort is higher risk than the previous 2014 study.

However, the studies included in the 2014 review had varying exclusion criteria such as excluding any pregnancies with an IUD or congenital anomalies. Two of the studies also only included women with a parity of 1 during the twin pregnancy. These exclusions could explain why the subsequent preterm birth rate in this study was found to be higher than previous findings, however further evaluation of this risk would have to be undertaken.

This cohort study found that 37% of the women who had a history of a previous preterm birth then went on to deliver the twin pregnancy before 34 weeks. Schaaf et al (129) found in 2012, a much higher rate of 56.9% of preterm twin pregnancy in mothers with a history of preterm birth. However, this study defined a preterm twin birth as <37 weeks, which could explain the much higher rate and discrepancy between the two findings. Similar studies classing preterm twin birth as <37 weeks have found the rate to be 16%-69% (184, 185).

A 2009 study found a subsequent preterm twin rate of 45.2% in mothers with a previous history of preterm birth when classifying the twin pregnancy preterm at <35 weeks gestation (184). These results are more in line with this current study's findings of 37%. When looking at twin pregnancies born <35 weeks as opposed to <34 weeks in this current cohort, 47% had a history of preterm birth, suggesting the different rate seen in previous literature is due to the difference in classification of gestational age of a twin preterm birth.

3.4.2b Parity

This study displayed that parity was a protective factor against preterm delivery, it prolonged the gestation of the twin pregnancy by 0.78 and 0.88 weeks (parity of 1 and 2), when analysing the data by gestational time. Multiparous women having a longer twin pregnancy gestation have been reported in other literature, with a 2011 study finding that nulliparous women (parity=0) had a <34 week preterm birth rate of 20.7%, with multiparous women's rate being 16.3% (186) although this result was not found to be statistically significant. In this same study, the difference in nulliparous and multiparous <37 weeks preterm birth was statistically significant. This current study's findings

found a nulliparous <34-week preterm birth rate of 25%, with multiparous rate of 17.1% (parity 1=18%, parity 2+=16%), which is comparable to other current literature.

3.4.2c BMI

Other literature has shown that women with a BMI >35 have an increased chance of a <34-week twin preterm birth with an odd ratio of 1.63 (1.30-2.05 confidence interval – p value 0.0001) (187). Another study only looking at DC twins found that obese women were more likely to deliver <34 weeks when compared to non-obese women (OR 1.65) (188). A similar DC study found an increased preterm birth rate in women with a BMI or ≥30 (P value <0.01) (189). This current study only identified statistical significance for the effect of BMI on MC pregnancies, with each increase in BMI by 1kg/m2 to decrease gestation time by 0.03 weeks (when comparing maternal BMI across two pregnancies). In DC pregnancies, this same difference was found to decrease gestational time by 0.01 weeks but this result was not statistically significant.

3.4.2d Maternal age

Extremes of maternal age have been reported to increase the risk of a preterm birth in singleton pregnancies but there have been mixed results for twins. A variety of studies have found than an increasing maternal age increases the risk of a PTB(190), with some others identifying this only for late PTB (34-36+6 weeks)(191).

There have been contrasting results with other studies showing twins have lower rates of preterm birth in mother with advanced age. A 2008 study found that women over 35 years had a statically lower rate of preterm twin birth than women age 25-29 years (192). A recent meta-analysis found that advanced maternal age was associated with a lower rate of PTB than with women under 35 years (PTB = <37 weeks), however no significant difference was seen in PTB rates between the two in births <32 weeks (193).

This current cohort study found a small but significant increase in the gestation of a twin pregnancy for every yearly increase in maternal age from 16 years old. Using this current study results, a 10-year increase in maternal age would increase the gestation of the twin pregnancy by 2.1 days – a very small impact. The effect of maternal age on PTB was only found during univariable analysis, not multivariable analysis which could suggest other variables that are connected with an increased maternal age e.g. a DC pregnancy and increased parity, could be responsible for this finding.

The incidence of preterm birth being higher in the extreme low end of maternal age (<20 years) has been linked to several different factors. Younger mothers are less likely to seek healthcare in the first trimester/entire pregnancy and more likely to come from a lower socio-economic background and have lower levels of maternal education (194). Despite this, an increased incidence of PTB has been found in mothers <20 years after adjusting for these factors (195, 196).

3.4.2e Chorionicity

Previous data clearly shows that MC pregnancies are at much higher risk of PTB than DC (197-199), a trend also described in this cohort study with 35% of MC and 16% of DC pregnancies born <34 weeks. The increase in MC pregnancies can be seen across a large range of PTB gestations (197). For this study, a MC pregnancy was found to reduce gestational length by 1.79 weeks, the only larger impact variable was a history of PTB. Some of this difference can be attributed to vascular complications that occur in MC pregnancies due to the MC unique complications of TTTS or sFGR secondary to the sharing of a placenta.

3.4.3 Implications

These results show that the maternal variables that can predict preterm birth are similar across both MC and DC pregnancies, with each variable having slightly more impact on gestational time in DC twins. There is limited current literature reporting preterm birth predictors via chorionicity, if a study does split by chorionicity, it normally reports on only the DC pregnancies.

Currently, the 2019 NICE guidelines on preventing preterm birth do not offer any recommendations for the prevention of twin preterm birth. They state that a twin mother should not be offered arabin pessary, bed rest, cervical cerclage or oral tocolytics to prevent spontaneous preterm birth. Intramuscular progesterone should not be offered as it has previously shown no clinical benefit, and produced unpleasant side effects for the mothers. NICE has not made any recommendation on the use of vaginal progesterone due to lack of evidence, but with new emerging evidence expected (66).

3.4.4 Strengths and limitations

A major strength of this study is that it is a large cohort of twin pregnancies with a good split of both MC and DC pregnancies. This relatively large sample size gave a good overall image of the likely overall picture seen in the general population and its findings are likely to be applicable to the overall twin population.

A key strength was that we had low missing data, with chorionicity and gestational weeks being collected for all of the 1584 pregnancies. This resulted in no pregnancies having to be excluded for key missing data (gestational weeks and chorionicity), the largest amount of missing data came from smoking status of the mother and use of ART.

Another strength of this study is that any pregnancy that had been referred to LWH from outside the area was excluded, due to the potential difference in care and management these twin pregnancies may have received. LWH is a tertiary unit that many complicated pregnancies are referred to. By including these pregnancies, this potentially may have artificially inflated the preterm birth rate seen in this study, and would have been less representative of the UK twin population.

Some limitations of this study include its retrospective design, which relied on historical data entry accuracy. The process of collecting data from multiple sources will have reduced the impact of this potential limitation.

Another limitation of this study is the lack of maternal health data collected, only BMI, previous births and previous cervical surgery was collected. Iatrogenic births are responsible for approximately 25% of births, some of these may be due to maternal factors. Including additional maternal health data may further strengthen the findings of this study.

Other limitations of this study include lack of smoking data collected as it is a known risk factor for PTB in singletons (200), it would be useful data to collect to see if smoking has the same impact on a twin pregnancy.

3.5 Conclusion

This data highlights that gestational length of a twin pregnancy is decreased by monochorionicity and a history of a previous <37-week preterm birth. BMI was also found to have a small negative impact on gestational length in MC pregnancies. A multiparous twin pregnancy was shown to have

an increased gestational length than a nulliparous twin pregnancy, with the gestational length being longer with a parity of 2 or more compared to 1.

Chapter 4 Discussion

This thesis aimed to report on predictors of pregnancy loss and preterm birth in multiple pregnancy. The data has primarily shown that MC twins are born earlier and have a higher rate of miscarriage and PTB than DC twins. It has also displayed that parity, BMI and previous preterm birth all play a role in the gestation of a subsequent twin pregnancy, either reducing or lengthening the gestational weeks at delivery. Overall, this data confirms that a twin pregnancy is a high-risk pregnancy for adverse pregnancy outcomes, especially pregnancy loss and preterm birth.

Multivariable analysis found that the maternal factors of parity and previous history of preterm birth had very similar effects on gestational time in both MC and DC twins. History of a previous preterm birth (<37 weeks) had more impact on the DC pregnancy than the MC pregnancy, seen when analysing the data by gestational time and ≥34 weeks (-2.36 Vs -2 and -1.56 Vs -1.33 weeks). With similar variable effects and the removal of TTTS cases still resulting in a higher preterm birth rate in MC twins, suggests that there are another number of other variables that result in this increased preterm birth rate seen in MC twins.

4.1 Predicting preterm birth

The data presented in this thesis has shown a <34-week preterm birth rate of 35% and 16% in monochorionic and dichorionic twins, with a combined preterm birth rate of 20.8% across both chorionicities, which is in accordance with previous literature (201). The preterm birth rate commonly reported in singletons is much lower at 5.3%-7.1% (202, 203) with the <34 week being as low as 0.9%-2.1% (201, 204).

These data have highlighted that twins are at much higher risk of being born preterm than a singleton pregnancy, despite making up only 2.6% of all births, twins represent approximately 12.2.% of all preterm births (p value 0.05) (205). With the <34-week preterm birth rate in twins being higher than the <37-week preterm birth in singletons, correlates with twins being at increased risk of adverse neonatal outcomes associated with prematurity such as respiratory distress syndrome, severe intraventricular haemorrhage and low Apgar scores (205).

This study found some significant preterm birth indicators in the twin population.

One predictor was a history of PTB in an antecedent singleton pregnancy, which was found to decrease the gestation of the twin pregnancy both in MC and DC twins, with a slightly more negative impact seen in DC twins. This is comparable to existing literature which has reported a shorter gestation if the mother has had a previous PTB (184). Although this effect has been previously reported in twin pregnancy, we believe we are the first to identify the impact of a previous preterm birth on gestation at delivery in both monochorionic and dichorionic twin pregnancy. The explanation of this increased negative impact of a previous PTB seen in a DC pregnancy is not known, and with a cohort of 103 women with previous PTB, additional research with a greater cohort size is needed to fully assess this finding.

Another significant predictor was the pregnancy being MC. MC pregnancies are known to have unique complications due to the vascular connections through their shared placenta, increasing their chance of adverse pregnancy outcomes, due to TTTS etc. These also increase the chance of a PTB (197, 206). In this cohort, after the removal of the 32 TTTS cases, the MC preterm birth rate was still increased at 29.2% compared to the DC cohort, suggesting MC pregnancies are still at higher risk of PTB in the absence of MC specific complications, a finding seen in other studies when classifying PTB as <34 weeks (206).

This study also reported that the difference in chorionicity also had a major impact on fetal loss <24 weeks, with the risk of loss of at least one fetus before 24 weeks more than two times higher in an MC pregnancy than a DC pregnancy (RR 2.42 CI 1.45. 4.04). The same increased risk was found in both loss of one fetus and loss of both fetuses <24 weeks gestation, although only the loss of one fetus <24 weeks was found to be significant.

Previous studies have found that the death of one twin in a MC pregnancy is more likely to lead to death of the co-twin, when compared to a DC pregnancy (207) due to vascular complications secondary to a shared placenta. This would suggest that if the death of one twin is significantly different between MC and DC pregnancies, the death of both twins should also be significantly different. However, this study did not find a statistically significant different between death of both twins. This cohort study did not record the cause of the fetal loss <24 weeks, which may have given more insight as to why the death of both twins was not significant between MC and DC twins. Additionally, only 15 pregnancies lost both fetuses which may be a too small cohort to find a significant link.

A younger maternal age was also found to be associated with a shorter gestation than an older mother in this study, with the gestation increasing by 0.03 weeks (0.21 days) for every yearly

increase from 16 years of age. The effect of maternal age on a twin pregnancy has be reported with conflicting results, with similar results of higher preterm birth rates seen in younger mothers being reported in a 2019 study but with the result found to not be statically significant (208). A finding of a lower twin PTB rate in women >35 years compared to women <35 years was reported in a recent meta-analysis (193), although like with many studies, PTB was classed as <37 weeks gestation. In this cohort study, maternal age being a significant risk factor for PTB was only found during univariable analysis, and was not found to be significant when separating the data by chorionicity. The small impact found in this study agrees with previous published data but suggests that maternal age may not be an accurate standalone predictor of PTB, but may be useful when combined with other PTB risks.

Having a parity of 0 was shown to have a negative impact on the gestational age, with having at least one pregnancy >24-weeks previously, associated with an increase in the gestational age at delivery of the subsequent twin pregnancy. This has been widely reported in the literature previously (136, 209, 210) will multiparity showing a protective factor against PTB. Being multiparous had a positive effect with similar lengthening on the gestation of a MC and DC pregnancy despite a significant difference seen in parity between the two groups. A previous study found primiparous twin mothers had significantly higher rates of preeclampsia and hypertension (211), both of which can contribute to PTB. The association between multiparous mothers and lower risk of PTB could be explained as in most cases as parity increases, maternal age also increases; another protective variable against PTB. We know that a previous PTB increases the risk of a subsequent PTB in singletons (212), so it is unsurprising that a previous term birth decreases the risk of a PTB in the subsequent pregnancy.

However, this finding was still reported after multivariate analysis, in which maternal age would've been adjusted for. Therefore, multiparity can be reported as an independent protective factor against twin PTB.

This study identified that BMI had a significant effect on the gestation of a MC twin pregnancy with the gestational length decreasing by 0.03 week (0.21 days) for every 1 increase in booking BMI, so we would expect a woman with a BMI of 35 to have a gestation 2.1 days shorter than a woman with a BMI of 25. Other literature has reported that a high BMI can increase the chance of PTB, however this literature is often not reported by chorionicity (213). A 2010 Japanese study only looking at DC twins found an increased risk of PTB <32 weeks in obese mothers (189). Interestingly this current study found that BMI only had a significant impact on MC pregnancies and not DC pregnancies.

This difference could be explained by BMI having more of an impact in younger mothers, with the average maternal age in an MC pregnancy being just over 2 years less than the DC cohort despite the average BMI being similar across the two groups. A 2019 study in singleton pregnancies found that white women <20 years and black women <30 years, maternal obesity was associated with an increased risk of PTB (214), with maternal obesity having a positive effect on PTB in older mothers. For the true effect of impact of BMI on twin PTB risk to be seen, MC and DC cohorts with similar maternal age may have to be studied.

4.2 Twin preterm birth

4.2.1 Prediction:

In singleton pregnancies, cervical length and fetal fibronectin (fFN), alongside PTB risk factors, are currently used as measurements for the prediction of PTB in singletons. The prediction of preterm birth in a twin pregnancy is not as clear and the evidence is not as strong.

Measuring cervical length has been assessed in various studies. One study concluded that a cervical length of <30mm between 16-20 weeks gestation was not a good predictor of PTB in a twin pregnancy. This study included low risk, asymptomatic (no signs of preterm labour) twin pregnancies (215).

A 2020 cohort study concluded that serial cervical length measurements were better at predicting twin PTB than a single mid-trimester measurement but still only predicted around 60% of preterm births (216). Meta-analysis has shown that the efficacy of cervical length PTB prediction depends on the gestational age at measurement, with <28-week PTB best predicted by a measurement at <18 gestational weeks (cervical length <30mm). A PTB between 28+1-36+0 weeks was found to be best predicted by a >22+0 gestational week screening (217).

This supports the 2020 cohort study conclusion that multiple cervical length assessments may be needed to more accurately predict twin PTB. Despite this, the evidence for cervical length screening in a twin pregnancy for predicting PTB is mixed, with different studies including or excluding specific cohorts of twin pregnancies. PTB prevention in singletons is recommended for pregnancies that have multiple PTB predictors, such as short cervical length and history of previous PTB (66). Combining

cervical length screening with another predictor of PTB, such as previous history of PTB or low maternal age, could lead to more accurate prediction and an increased efficacy.

A fFN level of >50 ng/mL is considered positive in most current literature. A 2020 analysis found that a positive fFN level increased the odds of a twin PTB, at <28 weeks (OR 12.06), <32 weeks (OR 10.03), <34 weeks (OR 6.26) and <37 weeks (OR 5.34), in unselected twin pregnancies (132). However, this analysis included studies that measured fFN levels at various gestations, so the optimum gestational age for measuring fFN is unknown. A recent 2020 prospective cohort study concluded that a fFN measurement between 22+0-27+6 weeks gestation was the most accurate time to predict <30-week twin preterm birth. The prediction was reported to be more accurate in women with an additional PTB risk factors, such as short cervical length (218) suggesting that for fFN to be used as a preterm birth predictor, the correct cohort must be measured. The current cohort study presented here did not collect any fFN levels due to the retrospective nature of the study and reliance on routine clinical data. It would be beneficial if future studies were able to more clearly define the position of fFN in the prediction of preterm birth in twin pregnancy. fFN levels taken in women with risk factors for a PTB could provide a new cohort for PTB prediction studies and lead to a new set of pregnancies to trial PTB preventions.

Studies looking at the link between deprivation and PTB has shown an increased incidence of PTB in the most deprived areas. A large publication looking at preterm birth in England and Wales found PTB rate to be 6.7% in the most deprived areas and 4.5% in the least deprived areas (using the Index of Multiple Deprivation) (219). This same link has been found in other countries with a 2020 German study finding higher a PTB in the most deprived areas (220). Due to the retrospective nature of this cohort study, no data on the women's socio-economic status and education level was collected, so the impact of deprivation on PTB rates is unable to be commented on in this study. However current literature has displayed that it could be an important predictor of PTB, concluding that the routine collection of this data could lead to further benefits in the prediction of PTB in the future.

4.2.2 Prevention:

There are several effective treatments to prevent preterm birth in singleton pregnancies and all have been tried in multiple pregnancy.

One intervention into the prevention of twin PTB is cervical cerclage. Recent cohort studies have shown a decrease in <34-week twin PTB in women with a cervical cerclage compared to without who have a cervical length measurement of <25mm. However, this decrease was further analysed and the benefit was only found in women with a cervical length of <15mm, the same statistically significant benefit was not found in the 16-25mm group (221).

A recent meta-analysis found conflicting studies about the efficacy of cervical cerclage in women with a cervical length of <25mm during a twin pregnancy. The use of a cervical cerclage was found to significantly prolong the twin pregnancy when the cervical length <15mm. However, this same increase in gestation was not found in women with cervical length 16-25mm or in women with a normal cervical length (>25mm) (222), suggesting a cervical cerclage is only beneficial to twin pregnancies that have more advanced signs of preterm labour (more advanced cervical changes). A more recent meta-analysis concluded that cervical cerclage showed the highest efficacy for being the best form of PTB prevention in women with cervical length <25mm, but this was concluded from a relatively small sample size so further trials would have to be undertaken to make this conclusion more robust (223).

Another mainstay of treatment for the prevention of PTB in singletons is vaginal progesterone
A recent meta-analysis concluded that vaginal progesterone showed no statically significant decreased in twin PTB when compared to expectant management , in unselected twin populations. In further sub group analysis of women with a short cervix (<25mm), there was also no decrease in <34 weeks PTB rates in the twin pregnancies (compared to expectant management) (223).

This is in comparison to a 2017 meta-analysis that reported vaginal progesterone significantly reduced the risk of <34 weeks preterm birth, and concluded that the number needed to treat to prevent one preterm birth was 6-12 women (224). However, a large amount of this data was drawn from one study leading to bias, suggesting more recent trials may give a more accurate result.
The current studies on prevention of twin preterm birth have all been undertaken on either unselected twin pregnancies or twin pregnancies with a short cervical length resulting in a lack of evidence for the use of any intervention. Use of PTB interventions in singletons is currently recommended in the NICE guidance for women with short cervical length but also other PTB risk factors such as history of PTB or mid-trimester loss (66).

The EVENTs trial concluded that the incidence of spontaneous <34 week PTB in twins was not reduced by treatment with vaginal progesterone, but post-hoc analysis did show there may be some benefit for women with a short cervix (<30mm). This trial did suggest that vaginal progesterone may play a role increasing the gestational weeks of <24 week pregnancy, changing late miscarriages to early PTB suggested by the higher late miscarriage rates seen in the placebo group (2.6% vs 4.4%) (225).

The STOPPIT-2 trial (226) involved a number of twin pregnancies from LWH recorded between 2015 and 2019, incorporating some twin pregnancies from this cohort. Due to this trial, a small number of women received an Arabin cervical pessary as a PTB intervention, however overall this represented very few of the pregnancies in this cohort. Therefore, most pregnancies in this cohort did not receive any preventative PTB intervention aside from normal twin pregnancy monitoring and care. The small number of pregnancies involved results in a lack of evidence being able to be collected for this specific intervention.

Currently NICE guidelines do not recommend any form an intervention for the prevention of PTB in a twin pregnancy. This is largely due to weak evidence (66).

The weak evidence could be strengthened by trials targeting a specific group of twin pregnancies that are at higher risk of PTB, rather than including all twin pregnancies. This cohort study identified specific variables that increased the PTB risk in a twin pregnancy such as primiparous, previous <37-week PTB, lower maternal age, higher BMI and MC chorionicity. New trials testing PTB interventions on these specific cohorts could provide more robust evidence and potential implementation of PTB preventative treatments in a twin pregnancy.

Screening and implementing a preventative treatment for twin PTB in only high-risk groups could be challenging due to the small number of cases likely to meet strict inclusion criteria, with the most significant factor in this cohort being previous PTB with only 104 pregnancies (6.6%). It is therefore likely that a national approach would be required to ensure enough twin pregnancies can be included in such a study.

4.3 Conclusion

This study confirms that there are recordable variables in a twin pregnancy that make it higher risk for PTB. Both fetal loss <24 weeks and early preterm birth are increased in monochorionic twins compared to dichorionic twins. By removing the MC pregnancies that had a diagnosis of TTTS, a vascular complication only seen in MC pregnancies, both the risk of fetal loss and preterm birth decrease, although the remaining MC twins still show higher PTB rates in comparison to DC twins.

Previous history of preterm birth, increasing maternal age and increasing BMI all reduced the gestation of a twin pregnancy in MC twin pregnancy. Prev PTB and maternal age reduced gestation in DC twin pregnancy. In both MC and DC twins a history of a previous PTB <37 weeks was the most significant risk factor for subsequent twin preterm birth. Previous term pregnancy with a parity of 1 or more was protective and increased the gestation at delivery in both MC and DC twin pregnancy.

However, there is little evidence on effective prevention of PTB in twins, and the incorporation of a screening test using maternal history will depend on the development of a proven effective treatment for the prevention of PTB. Using maternal risk factors found in this thesis to screen women into high or low risk groups for PTB could potentially allow further studies to be done on only these specific pregnancies, and to see if a stronger more established link can be made to explain why these pregnancies are at higher risk of PTB and to target interventions accordingly.

Most of the current evidence available for the efficacy of different methods for prevention of PTB in twins have used varying inclusion criteria or have targeted cohorts of twin pregnancies without regard to chorionicity. It is difficult to draw conclusions from this current data about what is an effective treatment due to conflicting or weak evidence. The maternal predictors found in this current study could provide a cohort of higher risk pregnancies, who could be then used in a trial to test the efficacy of prevention treatments. Positive results have been seen in women with a cervical length <15mm and treatment with cervical cerclage, showing that if a more accurate high-risk cohort of women are identified, there may be some potential benefit from existing PTB interventions. This result has been seen in singleton pregnancies when targeting women with a history of a previous PTB, but the current evidence is much further behind in twin pregnancies.

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