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Twin chorionicity-specific population birth-weight charts developed with adjustment for estimated fetal weight

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Short title: twin chorionicity-specific birthweight reference charts

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What are the novel findings of this work?
We developed novel estimated fetal weight (EFW)-adjusted population twin birthweight charts. The median birthweight for twins is consistently lower than singletons. Twin birthweight was consistently lower than the last recorded EFW. Therefore, it could not be assumed that median EFW and median birthweight are equal at any given gestation.

What are the clinical implications of this work?
The use of singleton charts in assessment of fetal growth in twins is controversial. This study presents EFW-adjusted twin birthweight charts, which shows the median birthweight for twins is consistently lower than singletons. The use of these novel charts may reduce misclassification and improve identification of growth restriction in twins.

ABSTRACT

Objective: To construct chorionicity-specific birthweight reference charts for dichorionic diamniotic (DCDA) and monchorionic diamniotic (MCDA) twin pregnancies incorporating estimated fetal weight (EFW) data in order to adjust for the relationship between suboptimal growth and premature delivery. An additional aim of this study was to determine if the inclusion of complicated twin pregnancies impacts the reference charts produced.

Methods: The Inclusion criteria were twin pregnancy of known chorionicity, known pregnancy outcome, last ultrasound scan within 14 days of birth which took place between 25 and 38 weeks (Analysis A). An analysis was also conducted excluding any pregnancies with complications recorded (Analysis B). The analysis makes use of previously published twin EFW reference ranges. A joint statistical model for EFW and observed birthweight for each pregnancy was created in order to estimate population birthweight reference ranges corresponding to the distribution expected were all pregnancies to deliver at any given gestation. It was not assumed that the median EFW was equal for any given gestation. The models were fitted using a Bayesian approach.

Results: We retrieved data on 1664 twin pregnancies, of which 707 DCDA and 241 MCDA pregnancies met the inclusion criteria. The estimate population median birthweight was similar to median EFW around 27 weeks but fell below the EFW values with increasing gestation to 156g lower in both DCDA and MCDA pregnancies at 35 weeks; this finding was confirmed by direct comparison of last EFW and birthweight values in each pregnancy.

When the analysis was repeated after excluding the complicated twin pregnancies, there was very little difference between the results obtained when comparing the median EFW across gestations, in Analysis A and those in Analysis B. The largest absolute difference in DCDA twins being a decreased median birthweight of 9g in the Analysis A cohort at 31, 32- and 33-weeks, when compared to Analysis B. The largest absolute difference in MCDA was greater showing an increased median birthweight of 25.3g in the Analysis B cohort at 25 weeks, when compared to Analysis A.

Conclusion: We established population reference chorionicity-specific birthweight charts for all twin pregnancies, corresponding to the range expected were all pregnancies to deliver at any given gestational age. In this population the median birthweight for twins is consistently lower than singletons and there is a variation in the median birthweight at different gestational ages for chorionicity.

BACKGROUND

Twin pregnancies accounted for 1.4% of all UK births in 2018 but are disproportionately at increased risk of perinatal mortality and morbidity [1], making it vital that these pregnancies are appropriately monitored. There is a particular risk of prematurity and fetal growth restriction [2,3]. Current evidence suggests that the use of singleton charts to monitor twin pregnancy is inaccurate, when used to assess for pathological growth in twin pregnancies [2–5], with previous evidence suggesting that twin specific EFW charts are more specific for identifying small-for-gestational-age (SGA) infants when compared to singleton charts, and performed just as well at identifying those at risk of stillbirths [6]. Despite this there are still no valid nomograms or birthweight charts that are routinely used for twin pregnancies [2]. This inadequacy resulted in the Global Twins and Multiples Priority Setting Partnership (PSP) including in their top ten research priorities for twin pregnancies the following question, “What are the expected growth patterns of SGA multiples?” and “how can we assess the growth of infant multiples and ensure they follow a satisfactory trajectory?” [7].

Evidence suggests that the median birthweight in twins is significantly lower than singletons, noticeable from around week 30-32 [3,4,8]. However, the reason for this difference in growth pattern remains debated. Firstly, twin-singleton birthweight divergence may be suggestive of a truly growth restricted infant, with studies suggesting that 2nd trimester growth is essentially in the same in twins and singletons [9,10]. However, it is in the third trimester that energy demands cannot be met and therefore represent a true placental insufficiency [11], with studies instead suggesting the use of individualised growth assessment [12] Secondly, twin pregnancies may just be smaller than singleton babies and therefore assessing their growth compared to singletons may be less specific [6].

There have been several birthweight reference charts generated for twins, charting the birthweight alongside the gestational age (GA) [13–18]. These studies opted to analyse actual observed birthweight. However, it is known that these reference charts have limitations due to the correlation between premature delivery and growth restriction and other pathological conditions [3]. In addition a number of these studies opts to assess birthweight differences between male and female twins rather than chorionicity [13,14,16,17]. Given these limitations, reference charts for birthweight have been developed in singletons that also make use of ultrasonographic data in order to include fetuses still in utero for estimation of the true weight distribution at any given GA [19].

A number of studies have published twin-specific growth and birthweight charts [2–4,6,18] however, their use in clinical practice remains controversial, We developed new population reference charts for birthweight in dichorionic diamniotic (DCDA) and monochorionic diamniotic (MCDA) twin pregnancies, incorporating estimated fetal weight (EFW) data in order to adjust for the relationship between suboptimal growth and premature delivery, similar to those suggested by *Nicolaides et al* [19]. The second aim of this study was to determine if the inclusion of complicated pregnancies impacts the overall reference charts produced.

METHODS

Study Population

We conducted a retrospective cohort study of twin pregnancies with clinic attendance between January 2000 and August 2019 at St George's University Hospital NHS Foundation Trust, London. The data were gathered from ultrasound and delivery database, collectively recorded on and identified searching electronic maternity records (ViewPoint version 5.6.26.148, ViewPoint bildverarbeitung GMBH, Wessling Germany and E3, Euroking Maternity Software Solutions Ltd, UK). The inclusion criteria were uncomplicated twin pregnancies of known DCDA or MCDA chorionicity; known pregnancy outcome; whose last ultrasound scan was within 14 days of delivery; who delivered between 25 and 38 weeks' gestation (**Analysis A**). Ethical approval was not required for this retrospective study of routinely collected anonymous data, as determined by local institutional review board guidance.

A second analysis, **Analysis B** was performed to include the following additional exclusion criteria; complicated twin pregnancies; fetal structural or chromosomal abnormalities; early neonatal death; intrauterine death of one twin; twin to twin transfusion syndrome (TTTS) or twin anaemia polycythaemia sequences (TAPS); and selective fetal growth restriction (sFGR). This additional analysis was performed to see if the exclusion of these high-risk pregnancies made a significant impact on the overall reference charts produced.

Study Variables

The chorionicity of the pregnancy was labelled, determined by the number of placentas, and the presence or absence of the lambda sign at the inter-twin membrane-placenta junction - as well as the inter-twin membrane thickness at the site of placental insertion in the chorion at 11-14 weeks. Alternatively, chorionicity can also be identified by the number of placentas and fetal gender after 14 weeks' gestation [20–22]. GA was determined in the first trimester, assessing the crown-rump length of the larger fetus in cases of spontaneous conception. If in-vitro fertilisation had taken place, dating was performed according to the oocyte retrieval date or the embryonic age from fertilization. After 14 weeks, GA was determined using the fetal head circumference of the larger fetus [22–24].

sFGR was defined as one twin below the tenth weight centile and a weight discordance of more than 25% or a single twin with a birthweight below the third centile [25], twins were labelled in this study as sFGR using the singleton standard reported by *Poon et al.* and twin chorionicity specific standard reported by *Ananth et al.* [18,26]

This analysis focuses on EFW obtained less than 14 days before birth, EFW was calculated using the formula first published by Hadlock *et al.* [27]

$$\text{Log10(EFW)} = 1.326 - 0.00326*AC*FL + 0.0107*HC + 0.0438*AC + 0.158*FL \quad (1)$$

The ultrasound studies were performed by experienced sonographers or clinicians trained in fetal medicine and measurements for the purpose of EFW were obtained following the clinical guidance presented by ISUOG [20,28,29].

Statistical analysis

The analysis makes use of the twin EFW reference ranges reported by Stirrup *et al.* [4,30], with model parameters derived from the prior study treated as fixed and known. To adjust for gaps of up to two weeks from last EFW until delivery, a predicted EFW was generated on the date of delivery for each twin. This was achieved by calculating the equivalent percentile value for the GA at delivery (termed 'shifted EFW'). Pregnancies in which either twin showed a last recorded EFW with z-score of > 4 or < -4 were excluded from the model, accounting for any data errors or pathological conditions.

A joint statistical model for the shifted EFW values and the observed birthweight for each pregnancy, on the log 10 transformed scale was created. The four observations in each pregnancy were modelled as a multivariate normal distribution, with the mean and the marginal variance and covariance for the shifted EFW values at delivery GA determined according to the model reported by Stirrup et al. [30] The birthweight variance was modelled as a multiple (τ) of the marginal EFW variance, with the scaling factor allowed to vary with GA at delivery;

$$(\tau_i = \exp(\tau_1 + \tau_2 * GA_i))$$

Correlation parameters were defined for the association between EFW and birthweight between and within each twin, and between the two birthweights for each pregnancy. The median birthweight at delivery was not assumed to be equal to the median EFW for any given GA but was instead allowed to deviate as a quadratic function of GA, (with three β parameters and the quadratic term multiplied by 0.01 for numerical stability). The model was fitted using a Bayesian approach implemented in the Stan statistical software [31], with results reported as the posterior mean and 95% credibility intervals (CrI). The τ and β parameters were assigned a weakly informative standard normal prior, and the correlation parameters were assigned uniform priors over the interval [-1,1]. The median and variance of Log10(BW) as a function of GA were calculated based on the posterior distributions of the β and τ parameters, conditioned on the observed data, in combination with the parameters of the published EFW model.

The objective of the study was to establish population reference ranges for twin pregnancies, rather than normal ranges, therefore we included all pregnancies in our final review as did Nicolaides et al.[19] when creating birthweight references for singletons. The statistical approach described by Nicolaides et al. was considered for the present study. However, it was found that the BW of twin pregnancies were on average consistently lower than the last recorded EFW. These discrepancies were present even if the EFW was recorded close to delivery, a consistent finding with previous literature [32]. Therefore, it could not be assumed that median EFW and median birthweight are equal at any given GA. This was the rationale for fitting a joint model for birthweight and the EFW data in which the median for the two variables could differ according to GA. We aimed to estimate birthweight reference ranges for the hypothetical situation that all pregnancies were to deliver at any given GA, which would help to adjust for the fact that growth restriction and timing of delivery are likely to be associated.

RESULTS

We retrieved data on 1,664 twin pregnancies. Following the inclusion criteria, 17 MCMA pregnancies were excluded along with 36 excluded for unknown chorionicity, 5 because GA at delivery was missing, 8 because birthweight was not recorded for both twins, 17 because GA at delivery was <25 weeks, 142 because GA at delivery was >38 weeks and 491 because the last ultrasound scan was missing or greater than 14 days before delivery. This left 707 dichorionic diamniotic (DCDA) and 241 monochorionic diamniotic (MCDA) twin pregnancies in **Analysis A** (Figure 1a)**.** Information regarding maternal demographics is reported in Table 1*.*

Analysis A

Of 707 DCDA twin pregnancies, 15 pregnancies were excluded from the analysis because of an EFW Z score >4 or <-4 (n=692) (Figure 1a*)*. The reference values resulting from the fitted model are given in Table 2*.* In addition, a plot of z-scores calculated for observed birthweight values using the newly defined reference standard according to GA at delivery is presented in Figure 2a. It is noted that the observed birthweight values are on average lower than the expected population average (i.e. if all pregnancies were to deliver at any given GA) until around 36 weeks' gestation.

Of 241 MCDA twin pregnancies, 12 pregnancies were excluded from the analysis (Figure 1a) because of an EFW z-score >4 or <-4 (n=229). The reference values resulting from the fitted model are shown in Table 3. A plot of birthweight z-scores calculated using the newly defined reference standard according to GA at delivery is presented in Figure 2b*.* The observed birthweight values are on average lower than the expected population up until around 36 weeks' gestation.

The fitted birthweight reference standards for both DCDA and MCDA twin pregnancies have a median value below that for EFW for GA beyond around 30 weeks. Over-estimation of the birthweight by the EFW for EFW values above and around 2000g can be confirmed by plotting either the shifted EFW values against birthweight (Figure 3a) or the observed EFW against birthweight only for those with an interval to delivery ≤ 2 days (Figure 3b)

Furthermore, the birthweight and last EFW before delivery have been plotted onto the EFW reference charts [30] with the DCDA pregnancies shown in Figure 4 and 5 and MCDA in Figure 6 and 7, respectively.

Analysis B

Of the total 707 DCDA twin, 94 additional pregnancies were excluded from this further analysis due to various complications, diagnosed using the ISUOG twin guidelines [20] (Figure 1b, Supplementary Table 1) and an additional 13 because of an EFW z-score >4 or <-4 (n=600). The reference value results from the fitted model are given in Table 4. A plot of BW z-scores calculated using the newly defined reference standard according to GA at delivery is presented in Figure 8a. The observed birthweight values were on average lower than the expected population average (i.e. if all pregnancies were to deliver at any given GA), up until around 36 weeks' gestation.

Of the total 241 MCDA twin pregnancies, 44 additional pregnancies were excluded from this further analysis due to various complications (Supplementary Table 1) and an additional 6 because of an EFW z-score >4 or <-4 (n=191). The reference value results from the fitted model are given in Table 5. A plot of birthweight z-scores calculated using the newly defined reference standard according to GA at delivery is presented in Figure 8b. As for DCDA twin pregnancies, the observed birthweight values were on average lower than the expected population average up until around 36 weeks' gestation.

The fitted birthweight reference standards for both DCDA and MCDA twin pregnancies have a median value below that for EFW for GAs beyond around 30 weeks. Over-estimation of the birthweight by the EFW for EFW values above around 2000g can be confirmed by plotting either the shifted EFW values against birthweight (Figure 9a) or the observed EFW against birthweight only for those with an interval to delivery of ≤2 days (Figure 9b)

It should be noted that when comparing the median EFW across gestations, there is very little difference between the results obtained in Analysis A and those in Analysis B. The largest absolute difference in DCDA twins being a decreased median birthweight of 9g in the Analysis A cohort at 31 (PD, 0.6%), 32- (PD, 0.5%) and 33- (PD, 0.5%) weeks' gestations, when compared to Analysis B. The largest absolute difference in MCDA was greater showing an increased median birthweight of 21g (PD, 2.5%) in the Analysis B cohort at 25 weeks, when compared to Analysis A.

In addition, there is considerable overlap between the Credibility Intervals in analysis A and B. For example, at 31 weeks GA for DCDA pregnancies the P50 was 1,563g (CrI 1,542g-1,585g) within analysis A, compared to 1,572g (CrI 1,552g – 1,593g) in analysis B. This overlap is similar for MCDA pregnancies. For example, at 25 weeks GA the P50 was 835g (CrI 769g – 917g) within analysis A, compared to 856g (CrI 772g- 947g) in analysis B.

The main analyses presented have been run separately with this group of pregnancies either included or excluded from the model, meaning that we had not directly estimated differences between the groups of pregnancies defined by inclusion or exclusion from Analysis B. However, we have now run an additional model for all DCDA and for all MCDA pregnancies, in which the 'included' and 'excluded' groups share a variance structure but the difference in median weight is explicitly estimated in relation to GA with quantification of statistical uncertainty. The results are presented in Supplementary Table 2, and do not show clear statistical evidence of an overall difference in weight between the 'included' and 'excluded' groups. There is some evidence of lower weights among the excluded cases, but there is not strong statistical evidence for this – this is due at least in part to relatively small numbers of pregnancies in the 'excluded' group.

We also note that the exclusion criteria for Analysis B relate to prenatal or early neonatal outcomes, and so do not necessarily predict how birth weight would predict longer term outcomes beyond the immediate neonatal period.

Histograms of birth weight Z-scores relative to the newly derived reference ranges for (a) the 692 DCDA pregnancies and (b) the 229 MCDA pregnancies included in Analysis A are shown in Supplementary Figure.

DISCUSSION

Summary of study findings

Using EFW and BW from a diverse population of twin pregnancies we have created a BW reference chart for DCDA and MCDA twins. When comparing the median BW value across gestations of our newly generated charts to singleton charts, differences were noted. In addition, the median values differ between MCDA and DCDA twins, highlighting the importance of chorionicity specific charts. Finally, we noticed that within our population the EFW was often overestimating the true birthweight of the twin.

Additionally, we found that when comparing analysis, A and B, for both DCDA (*Supplementary Table 2)* and MCDA (*Supplementary Table 3)* pregnancies, the exclusion of additional pregnancies made no significant difference to the reference ranges achieved. Therefore, we have decided to use analysis A as it would be logical to generate reference charts based on the entire population, including abnormal twins.

Interpretation of study findings and comparison with existing literature

We compared our percentiles to those of Nicolaides et al. [19], who created birthweight references for singletons from 95,579 pregnancies. The DCDA twin median birthweight was similar to Nicolaides' results, until 28 weeks. For example, at 25 weeks, the median weight for DCDA twins was 780g and 797g (PD, 2.18%) for singletons. At 28 weeks, the weights were 1135g and 1228g (PD, 8.2%), respectively. This absolute weight difference increased steadily, with weights at 38 weeks, 2661g and 3219g (PD, 21.0%) respectively.

MCDA twins started heavier at 25 weeks when compared to singletons (835g compared to 797g respectively (PD, -4.55%), although there is considerable uncertainty in our estimate (95% CrI, 769–917 g). This absolute weight difference between MCDA twins and singletons increased from week 28 (1131g and 1228g respectively (PD, 8.6%). This discrepancy at the earlier GA in the MCDA cohort, is likely due to the smaller sample size.

Beyond 28 weeks, both MCDA and DCDA twins had a marked reduction in size when compared to singleton pregnancies, a trend that is a consistent finding in literature [2–4,14]. In addition, a larger absolute difference in MCDA when compared to DCDA twins is noted.

Our analysis made explicit use of the EFW reference ranges published by *Stirrup et al.*[30]. We found that the median birthweight drops below the median EFW as pregnancy progresses. For example, assessed, at 27 weeks the estimate for the 50th percentile value of EFW in DCDA twins was 1011g, compared to our new median birthweight estimate of 1008g (PD, 0.3%). However, at 38 weeks the median EFW was estimated to be 2853g compared to an estimated median birthweight of 2661g (PD, 7.2%).

Finally, we also compared our estimated 50th birthweight percentile values to other twin birthweight studies that compiled reference ranges split by chorionicity [15,18]. When compared to the *Ananth et al*.[18] study, the largest difference between birthweight in DCDA twins occurred at 35 weeks' gestation. Our study showed a median birthweight of 2,198g compared to a median birthweight of 2,359g (PD, 7.3%). At 38 weeks this absolute difference was smaller, showing a birthweight of 2661g and 2753g (PD, 3.5%) respectively. For MCDA twins the largest discrepancy occurred at 33 weeks' gestation with our study showing a median birthweight of 1780g compared to 1980g (PD, 11.2%). However, by 38 weeks this discrepancy had decreased to 2624g and 2660g (PD,1.4%), respectively. In comparison, when we analysed our dataset to those of *Premkumar et al.* [15], our cohort showed a consistently larger median birthweight across all GA in both MCDA and DCDA pregnancies. The differences discussed above, could be as a result of population variance. However, it should be noted that both the data presented by *these studies*[15,18] are based on observed birthweight measurements.

Strengths and Limitations

Our sample size is substantial for a twin study and the population used was from a diverse tertiary care centre. Moreover, we only used one ultrasound scan per fetus, therefore it is assumed we have only used routinely collected data to establish these new charts.

Given the large study period, delivery protocols for each twin may have differed from the current NICE protocol, this may have impacted a small fraction of the total pregnancies, particularly those born before 2011. Maternal characteristics, fetal sex and their effects on fetal growth were not considered, similar to previous studies [2,13,14,16,17,19]. Furthermore, around 10% of our pregnancies were dated in the second trimester, which may have led to some inaccuracies. Finally, a caveat of this study is that the credibility intervals at points indicate substantial uncertainty. This is particularly important at earlier gestational ages and for MCDA twins, owing to the smaller sample size. MCMA pregnancies were excluded entirely.

Clinical and research implications

The birthweight charts described in this study add to the limited research in this area. However, the extent to which this approach could improve detection of fetal growth restriction needs to be tested in prospective studies. For example, a small infant, is defined as those who are below the 10th percentile [33]. When comparing our data to the WHO neonatal weight charts [34] the 10th percentile of our birthweight chart are lower than those presented in the WHO study. At 36 weeks' gestation the 10th percentile in our charts for DCDA and MCDA twins were *1969g (PD, 19.5%)* & *1877g (PD, 25.3%)* respectively, compared to *2352g* for singletons in the WHO charts. Moreover, when compared to the 10th percentile of *Nicolaides et al.*[19] which sampled a larger population, the absolute difference between our 10th percentile and singleton's 10th percentile increased, at 36 weeks the singleton 10th percentile for birthweight was *2439g* [19].

It is important to acknowledge that outcomes for twins are worse when compared to singletons, which may be a result of earlier GA birth or lower BW. However, it is not possible to rule out effects of other covariates on overall development. Reclassifying this group of infants may change the interventions received, leading to poorer outcomes, so prospective analysis is needed before implementation to minimise the risk of false negative results.

Contribution

We present a novel method to generate twin chorionicity-specific EFW-adjusted population twin birthweight charts. We have demonstrated substantial differences to equivalent charts in singletons and non-EFW adjust twin birthweight reference charts and that there is distinct difference between MCDA and DCDA specific birthweight charts. Observational studies and Randomised trials are required to evaluate the use of these charts in clinical practice and assess their ability to predict neonatal adverse events.

Declaration of interest: None to declare

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FIGURE LEGENDS

Figure 1. (a) Flowchart showing the exclusion criteria for **Analysis A.** The following exclusions occurred, 17 MCMA pregnancies, 36 for unknown chorionicity, 5 due to missing GA, 8 due to lack of data for both twins, 17 because GA at delivery was <25 weeks, 142 because GA at delivery >38 weeks and 491 because the last ultrasound scan was missing or greater than 14 days prior to delivery. In addition, 15 DCDA Twins were excluded due to a z-score <-4 or >4 and 12 MCDA twins were excluded due to a z-score of <-4 or >4.

Figure 1. (b) Flowchart showing the exclusion criteria for **Analysis B,** The following exclusions occurred, 17 MCMA pregnancies, 36 for unknown chorionicity, 5 due to missing GA, 8 due to lack of data for both twins, 17 because GA at delivery was <25 weeks, 142 because GA at delivery >38 weeks and 491 because the last ultrasound scan was missing or greater than 14 days prior to delivery. In addition, 13 DCDA twins were excluded due to a z-score <-4 or >4, additionally 94 were excluded due to pregnancy complications (*Supplementary Table 1*). 6 MCDA twins were excluded due to a z-score <-4 or >4 and 44 were excluded due to pregnancy complications (Supplementary Table 1).

Figure 2. Plot of the Z-scores of birth weights in (a) dichorionic diamniotic and (b) monochorionic diamniotic pregnancies, calculated using the newly derived birth weight reference standard in Analysis A. The blue line shows a LOESS smoothed estimate of the mean according to GA, with 95% CI

Figure 3. Plots of birth weight against (a) shifted estimated fetal weight in all 921 twin pregnancies included in Analysis A and (b) EFW in only those pregnancies with EFW within 2 days prior to delivery (n= 144 pregnancies). The line of equality is shown (black line), along with a linear regression line (blue line) with 95% CI (shaded area)

Figure 4. Plots of birthweight of dichorionic diamniotic (DCDA) twins onto EFW reference chart the second graph plots the birthweight of 'complicated' DCDA twins onto the previously published EFW reference chart (Median, 5th and 95th centiles shown as black lines). The blue line shows a LOESS smoothed estimate of the mean according to GA, with 95%CI shaded. Plotted cases of stillbirth include one recorded early neonatal death.

Figure 5. Plots of last recorded EFW of dichorionic diamniotic (DCDA) twins onto EFW reference chart the second graph plots last recorded EFW of 'complicated' DCDA twins onto the previously published EFW reference chart (Median, 5th and 95th centiles shown as black lines). The blue line shows a LOESS smoothed estimate of the mean according to GA, with 95%CI shaded. Plotted cases of stillbirth include one recorded early neonatal death.

Figure 6. Plots of birthweight of monochorionic diamniotic (MCDA) twins onto EFW reference chart the second graph plots the birthweight of 'complicated' MCDA twins onto the previously published EFW reference chart (Median, 5th and 95th centiles shown as black lines). The blue line shows a LOESS smoothed estimate of the mean according to GA, with 95%CI shaded. Plotted cases of stillbirth include one recorded early neonatal death.

Figure 7. Plots of last recorded EFW of monochorionic diamniotic (MCDA) twins onto EFW reference chart, the second graph plots last recorded EFW of ‘complicated’ MCDA twins onto the previously published EFW reference chart (Median, 5th and 95th centiles shown as black lines). The blue line shows a LOESS smoothed estimate of the mean according to GA, with 95%CI shaded. Plotted cases of stillbirth include one recorded early neonatal death.

Figure 8. Plot of the Z-scores of birth weights in (a) dichorionic diamniotic and (b) monochorionic diamniotic pregnancies, calculated using the newly derived birth weight reference standard in Analysis B. The blue line shows a LOESS smoothed estimate of the mean according to GA, with 95%CI shaded

Figure 9. Plots of birth weight against (a) shifted estimated fetal weight in all 791 twin pregnancies included in Analysis B and (b) EFW in only those pregnancies with EFW within 2 days prior to delivery (*n*= 121 pregnancies). The line of equality is shown (black line), along with a linear regression line (blue line) with 95%CI (shaded area)

Supplementary Information

Supplementary Table S1. The breakdown of the number of pregnancies excluded in analysis B due to complications

Supplementary Table S2. Estimation of the difference in median weight according to gestational age (GA) between cases excluded from Analysis in comparison to those included in Analysis B for dichorionic diamniotic (DCDA) and monochorionic diamniotic (MCDA) pregnancies.

Supplementary Table S3 Estimation of the difference in median weight according to gestational age (GA) between cases excluded from Analysis A in comparison to those included in Analysis B for monochorionic diamniotic (MCDA) pregnancies.

Supplementary Table S4. Summaries for the fitted analysis models and for the model used for comparison of the 'included in Analysis B' vs 'excluded from Analysis B' subgroups.

Figure S1 Histograms of birth weight Z-scores relative to the newly derived reference 911 ranges for (a) the 692 DCDA pregnancies and (b) the 229 MCDA pregnancies included 912 in Analysis A. Z-scores were calculated on the log10(birth weight) scale

Technical description of statistical model

Table 1. Demographic data of the study population split into dichorionic and monochorionic pregnancies.

Pregnancy Characteristics	**Dichorionic diamniotic twin pregnancies (n=707)**	**Monochorionic diamniotic twin pregnancies (n=241)**
Maternal age in years, median (IQR)	34 (30-37)	32 (28-35)
Gestational age at birth in weeks, median (IQR)	36.9 (34.7-37.4)	35.7 (33.0-36.6)
Maternal body mass index in kg/m^2, median (IQR)	24.5 (21.9-28.5) (n=477)	23.9 (21.6-27.9) (n=156)
Nulliparous, n (%)	349/654	124/227
Gestational Diabetes (%)	33 (4.7%)	14 (5.8%)
Gestational Hypertension (%)	14 (2%)	0 (0%)
IVF Treatment (%)*	40 / 105 (38.1%)	5/33 (15.2%)
Self-reported ethnicity, n (%)	(n=644)	(n=222)
• White	386 (59.9)	140 (63.1)
• Black	115 (17.9)	23 (10.4)
• Asian	74 (11.5)	28 (12.6)
• Mixed	4 (0.6)	3 (1.4)
• Other	65 (10.1)	28 (12.6)
Smoker, n (%)	33/684 (4.8)	14/231 (6.1)
• Missing, n (%)	23 (3.3)	10 (4.2)
Alcohol Intake during pregnancy, n (%)	41/687 (6.0)	17/234 (7.3)
• Missing, n (%)	20 (2.8)	7 (2.9)
Gender of fetus, n (%)		
• Male	707 (50.0)	227 (47.1)
• Female	707 (50.0)	253 (52.5)
• Indeterminate	0 (0)	2 (0.4)

*_Only those pregnancies definitively recorded as having IVF or not were included in this data.

Table 2. Birth weight reference values for dichorionic diamniotic (DCDA) twins (**Analysis A**)

	Log$_{10}$(EFW)		**EFW (g)**					**EFW (g) P50 95% CrI**	
GA (weeks)	**Mean**	**Standard deviation**	**P5**	**P10**	**P50**	**P90**	**P95**	**Lower**	**Upper**
25	2.892	0.06858	602	638	780	955	1011	743	818
26	2.949	0.06749	689	729	890	1085	1148	855	924
27	3.003	0.06643	784	829	1008	1226	1296	978	1040
28	3.055	0.06541	886	936	1135	1377	1454	1108	1163
29	3.104	0.06443	996	1051	1271	1537	1622	1247	1296
30	3.150	0.06352	1112	1172	1414	1705	1798	1392	1437
31	3.194	0.06271	1233	1299	1563	1881	1982	1542	1585
32	3.235	0.06201	1358	1430	1718	2063	2172	1697	1739
33	3.273	0.06146	1487	1565	1876	2249	2368	1857	1897
34	3.309	0.06108	1616	1701	2037	2439	2567	2017	2057
35	3.342	0.06090	1745	1836	2198	2630	2768	2181	2216
36	3.372	0.06094	1871	1969	2357	2821	2968	2343	2371
37	3.400	0.06121	1992	2097	2512	3009	3167	2497	2525
38	3.425	0.06172	2106	2218	2661	3193	3362	2635	2683

EFW: estimated fetal weight; P5: 5th centile; P10: 10th centile; P50: 50th centile; P90: 90th centile; P95: 95th centile

Table 3. Birthweight reference values for monochorionic diamniotic (MCDA) twins (**Analysis A**)

	Log_{10}(EFW)		EFW (g)					EFW (g) P50 95% CrI	
GA (weeks)	**Mean**	**Standard deviation**	**P5**	**P10**	**P50**	**P90**	**P95**	**Lower**	**Upper**
25	2.921	0.09652	580	629	835	1110	1204	769	917
26	2.967	0.09205	655	707	927	1215	1313	867	996
27	3.011	0.08782	736	791	1025	1328	1429	975	1083
28	3.053	0.08385	824	884	1131	1448	1554	1087	1178
29	3.095	0.08014	920	983	1245	1577	1686	1207	1284
30	3.136	0.07674	1022	1090	1367	1714	1828	1331	1403
31	3.175	0.07365	1133	1204	1497	1860	1978	1461	1531
32	3.213	0.07091	1250	1326	1634	2015	2138	1598	1668
33	3.250	0.06854	1373	1454	1780	2179	2308	1745	1814
34	3.286	0.06653	1503	1589	1934	2353	2488	1904	1965
35	3.321	0.06490	1639	1730	2095	2538	2679	2069	2122
36	3.355	0.06363	1780	1877	2264	2732	2881	2239	2290
37	3.387	0.06270	1925	2028	2441	2936	3094	2405	2477
38	3.419	0.06209	2074	2185	2624	3151	3319	2562	2691

EFW: estimated fetal weight; P5: 5th centile; P10: 10th centile; P50: 50th centile; P90: 90th centile; P95: 95th centile

Table 4. Birth weight reference values for dichorionic diamniotic (DCDA) twins (**Analysis B)**

	Log$_{10}$(EFW)		EFW (g)					EFW (g) P50 95% CrI	
GA (weeks)	**Mean**	**Standard deviation**	**P5**	**P10**	**P50**	**P90**	**P95**	**Lower**	**Upper**
25	2.892	0.06332	615	648	781	941	992	738	826
26	2.950	0.06279	703	741	891	1072	1130	852	932
27	3.005	0.06227	799	842	1011	1215	1280	977	1046
28	3.057	0.06177	903	950	1140	1368	1440	1111	1171
29	3.106	0.06131	1013	1066	1277	1530	1610	1252	1303
30	3.153	0.06091	1129	1188	1421	1701	1790	1399	1443
31	3.196	0.06058	1250	1314	1572	1879	1977	1552	1593
32	3.237	0.06036	1374	1445	1727	2063	2170	1706	1748
33	3.275	0.06028	1500	1578	1885	2252	2369	1865	1907
34	3.311	0.06037	1627	1711	2045	2444	2570	2024	2067
35	3.343	0.06065	1752	1843	2204	2636	2773	2186	2224
36	3.373	0.06115	1872	1970	2360	2827	2975	2345	2376
37	3.400	0.06189	1986	2092	2511	3014	3174	2496	2527
38	3.424	0.06288	2092	2205	2655	3196	3368	2629	2681

EFW: estimated fetal weight; P5: 5th centile; P10: 10th centile; P50: 50th centile; P90: 90th centile; P95: 95th centile

Table 5. Birth weight reference values for monochorionic diamniotic (MCDA) twins (**Analysis B)**

	$\mathbf{Log_{10}}$(EFW)		**EFW (g)**					**EFW (g) P50 95% CrI**	
GA (weeks)	**Mean**	**Standard deviation**	**P5**	**P10**	**P50**	**P90**	**P95**	**Lower**	**Upper**
25	2.932	0.09703	594	644	856	1138	1234	772	947
26	2.974	0.0923	666	719	943	1237	1336	870	1019
27	3.016	0.08784	745	801	1037	1344	1446	976	1101
28	3.057	0.08366	831	891	1139	1458	1563	1087	1193
29	3.097	0.07977	924	988	1249	1580	1689	1204	1294
30	3.136	0.0762	1025	109	1367	1712	1824	1327	1407
31	3.174	0.07296	1133	120	1494	1852	1969	1455	1532
32	3.212	0.07009	1249	132	1629	2003	2124	1591	1667
33	3.249	0.06758	1373	145	1773	2164	2290	1734	1812
34	3.285	0.06546	1503	158	1926	2337	2468	1890	1964
35	3.320	0.06371	1641	173	2089	2521	2659	2057	2122
36	3.354	0.06233	1786	188	2261	2718	2863	2234	2289
37	3.388	0.06129	1937	203	2443	2927	3081	2405	2481
38	3.421	0.06057	2095	220	2635	3150	3314	2564	2706

EFW: estimated fetal weight; P5: 5th centile; P10: 10th centile; P50: 50th centile; P90: 90th centile; P95: 95th centile

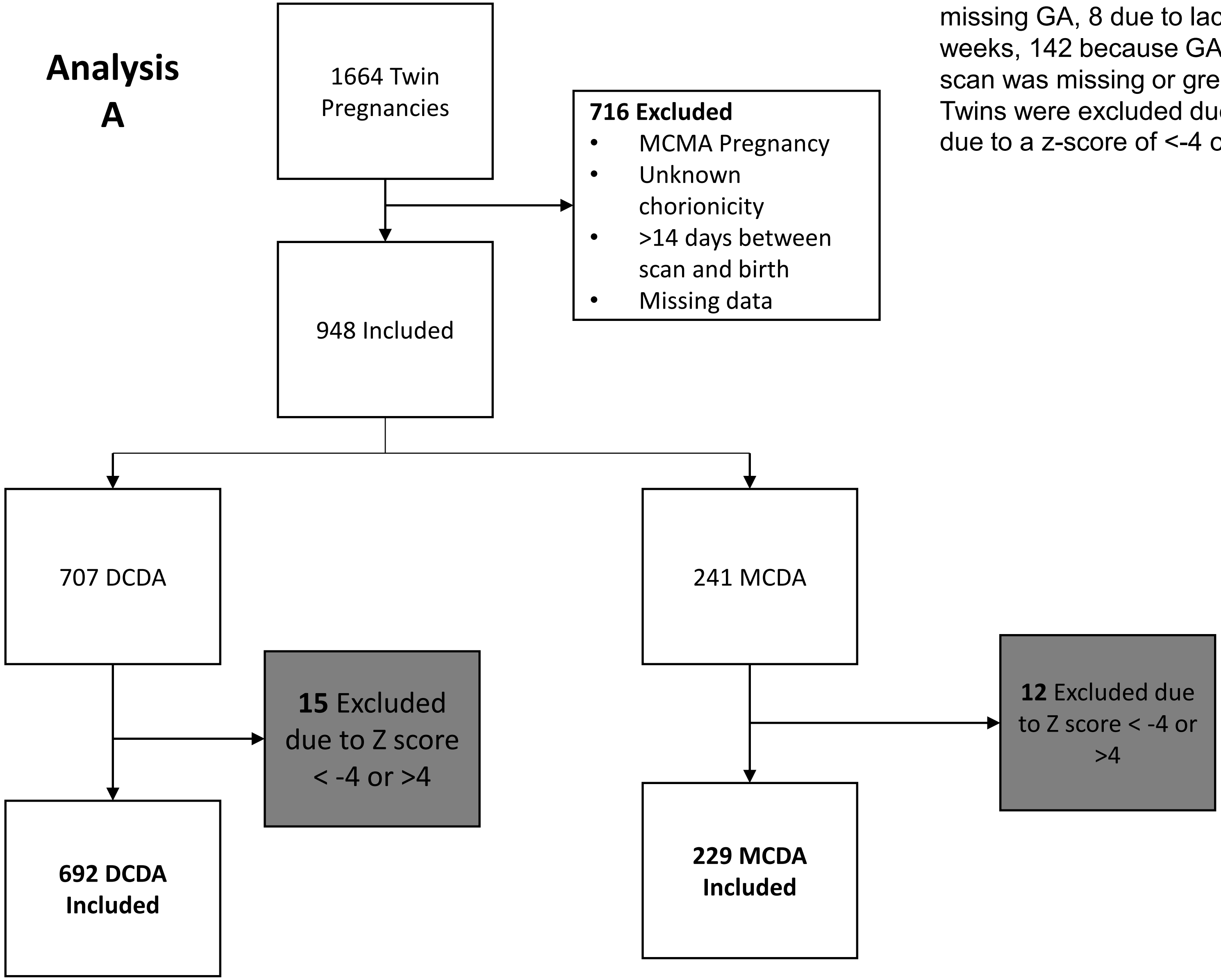


Figure 1. (a) Flowchart showing the exclusion criteria for **Analysis A.** The following exclusions occurred, 17 MCMA pregnancies, 36 for unknown chorionicity, 5 due to missing GA, 8 due to lack of data for both twins, 17 because GA at delivery was <25 weeks, 142 because GA at delivery >38 weeks and 491 because the last ultrasound scan was missing or greater than 14 days prior to delivery. In addition, 15 DCDA Twins were excluded due to a z-score <-4 or >4 and 12 MCDA twins were excluded due to a z-score of <-4 or >4.

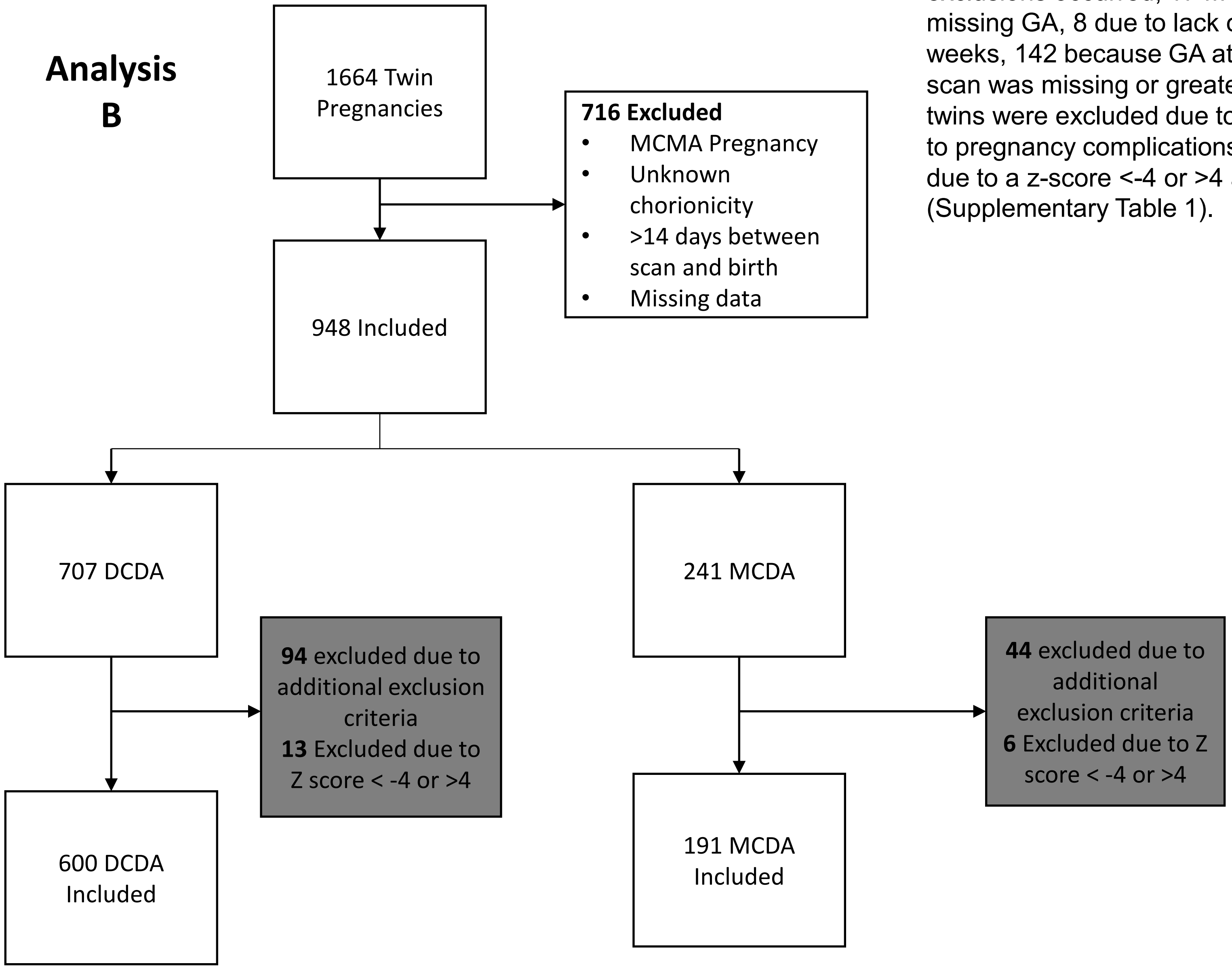
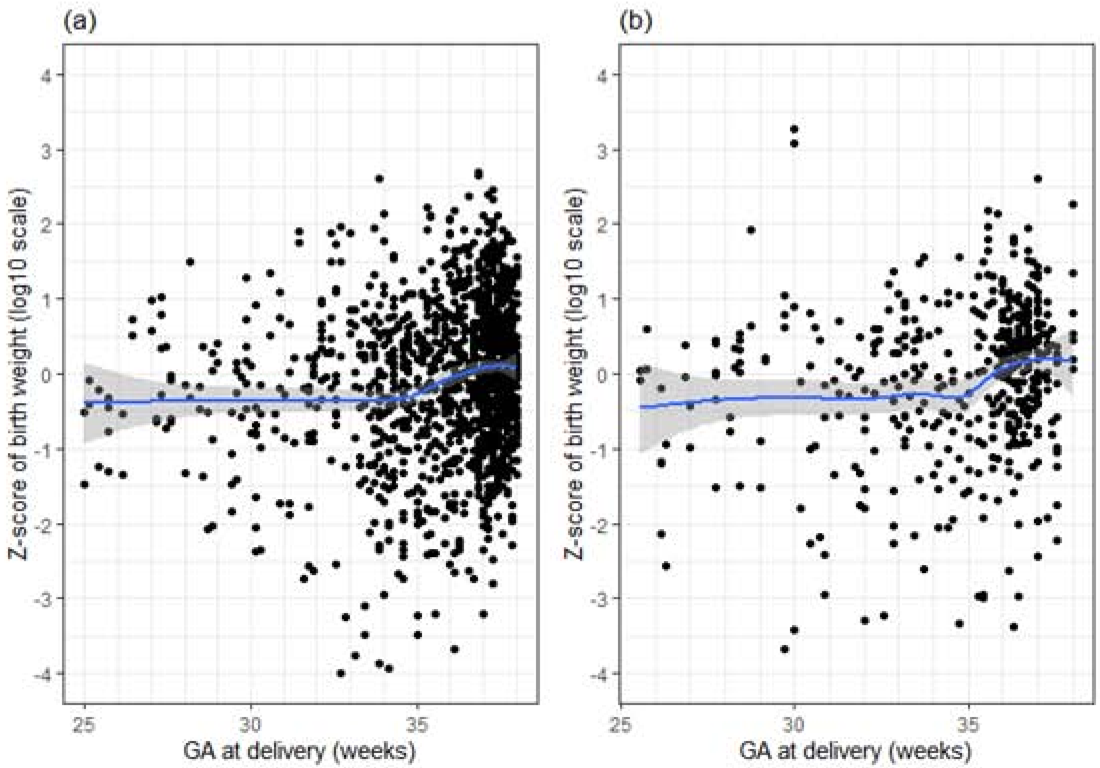


Figure 1. (b) Flowchart showing the exclusion criteria for **Analysis B,** The following exclusions occurred, 17 MCMA pregnancies, 36 for unknown chorionicity, 5 due to missing GA, 8 due to lack of data for both twins, 17 because GA at delivery was <25 weeks, 142 because GA at delivery >38 weeks and 491 because the last ultrasound scan was missing or greater than 14 days prior to delivery. In addition, 13 DCDA twins were excluded due to a z-score <-4 or >4, additionally 94 were excluded due to pregnancy complications (Supplementary Table 1). 6 MCDA twins were excluded due to a z-score <-4 or >4 and 44 were excluded due to pregnancy complications (Supplementary Table 1).



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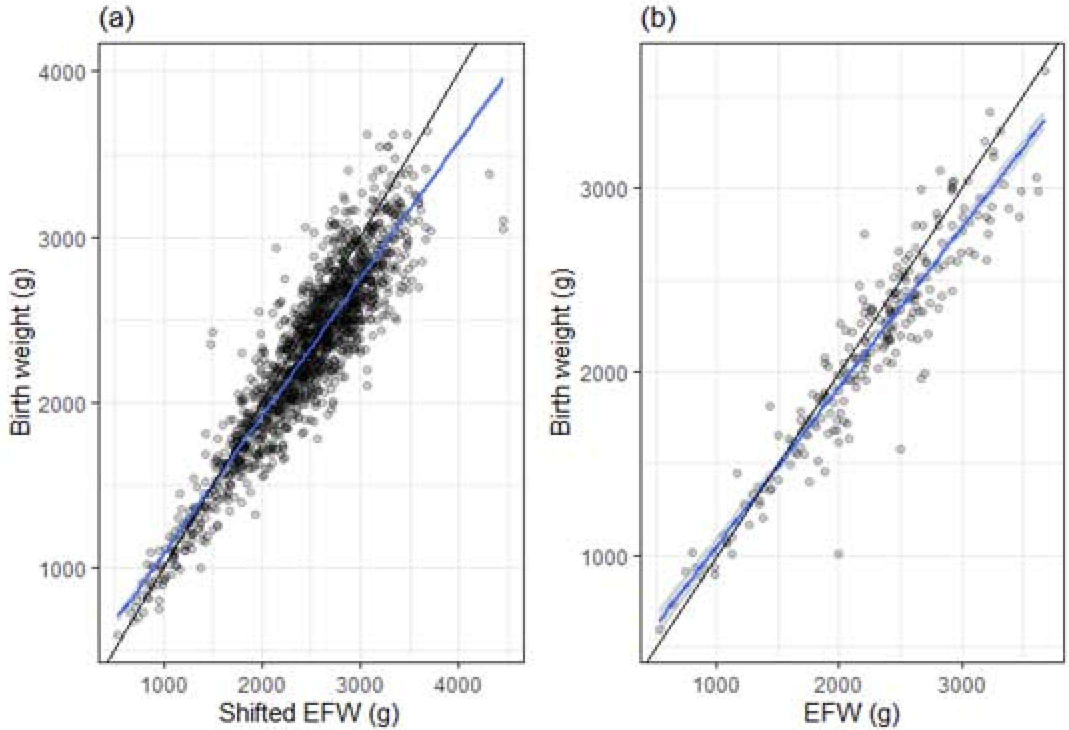
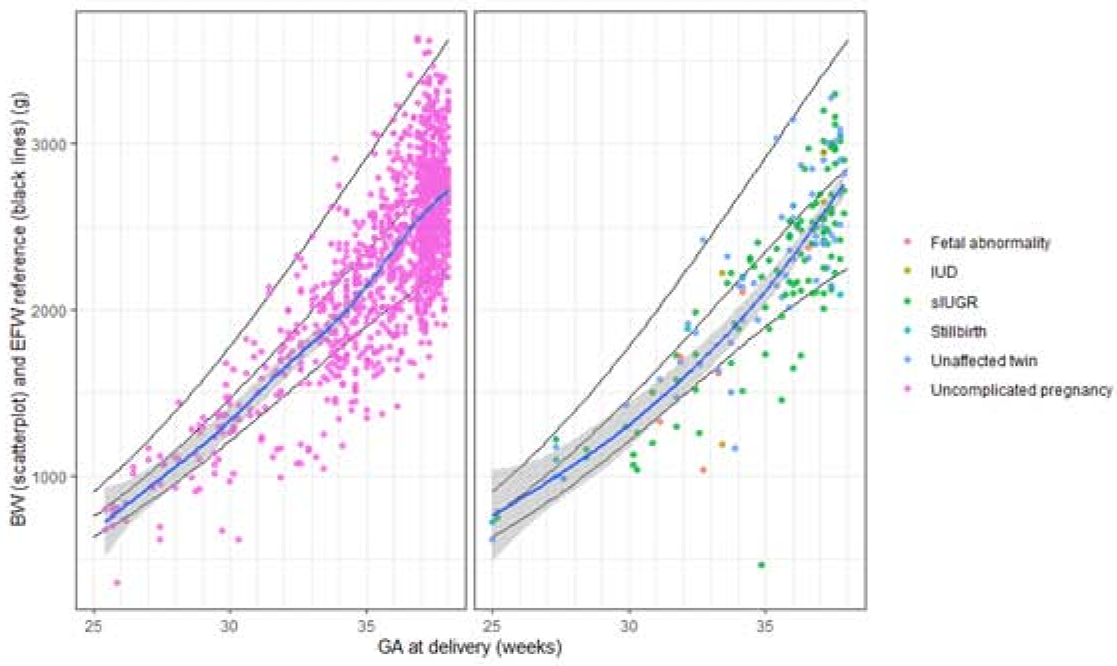
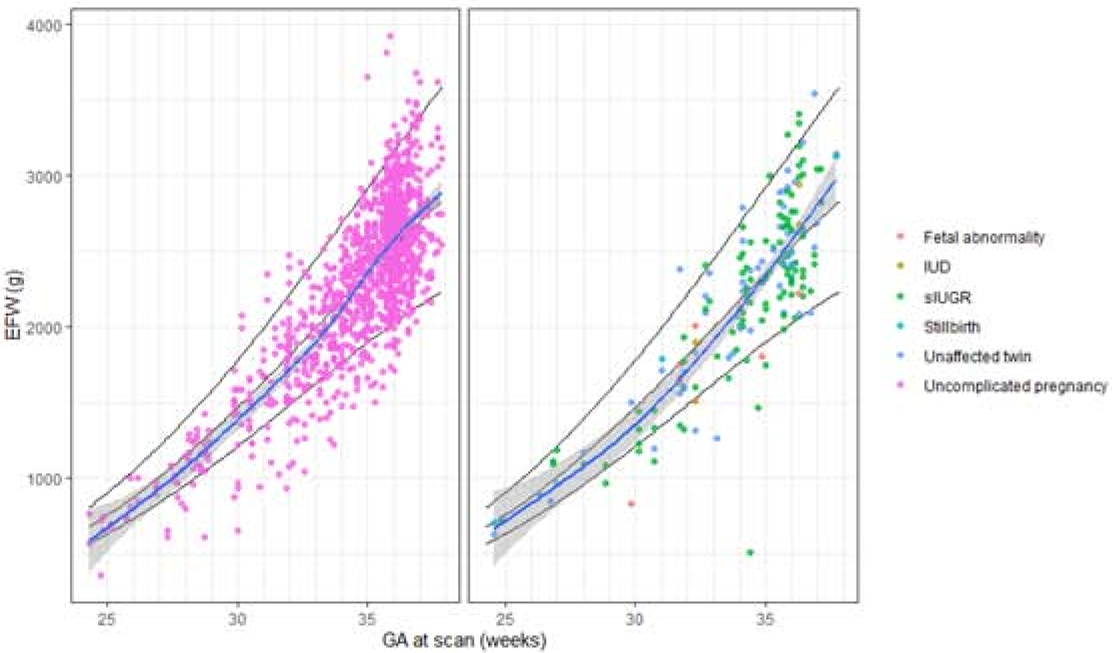


Figure 3. Plots of birth weight against (a) shifted estimated fetal weight in all 921 twin pregnancies included in the analysis and (b) EFW in only those pregnancies with EFW within 2 days prior to delivery (n=144 pregnancies). The line of equality is shown (black line), along with a linear regression line (blue line) with 95% CI (shaded area)



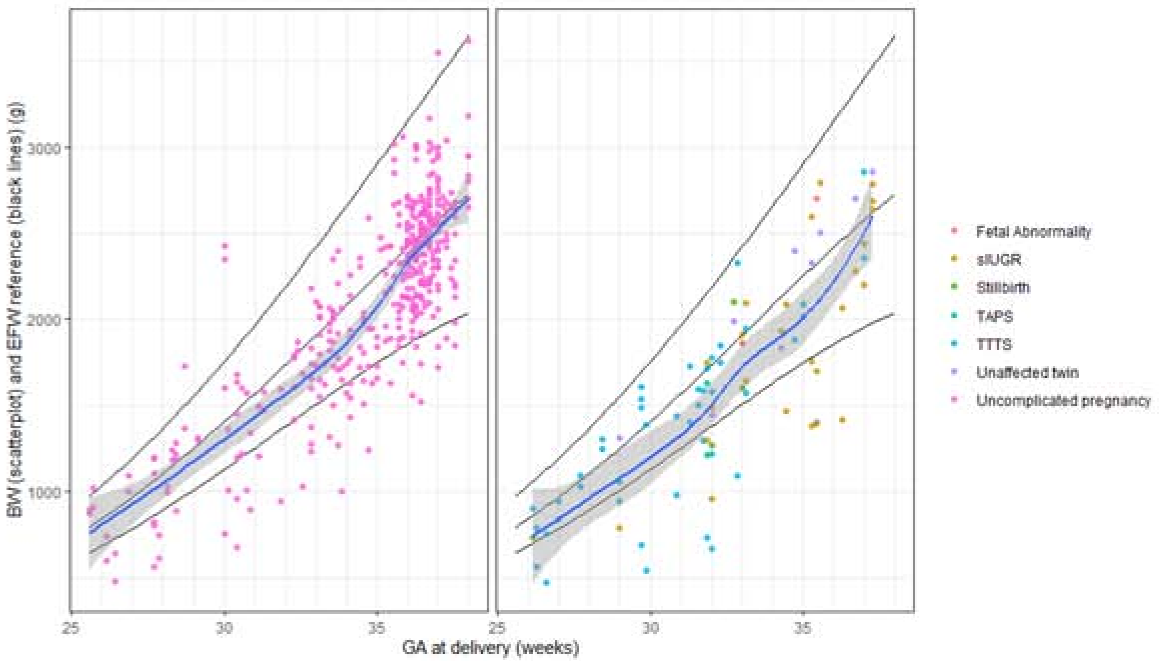
BW (scatterplot) and EFW reference (black lines) (g)
3000
2000
1000
25
30
35
25
30
35
GA at delivery (weeks)
Fetal abnormality
IUD
sIUGR
Stillbirth
Unaffected twin
Uncomplicated pregnancy

Figure 4



4000
3000
2000
1000
EFW (g)
25
30
35
GA at scan (weeks)
Fetal abnormality
IUD
sIUGR
Stillbirth
Unaffected twin
Uncomplicated pregnancy

Figure 5


BW (scatterplot) and EFW reference (black lines) (g)
3000
2000
1000
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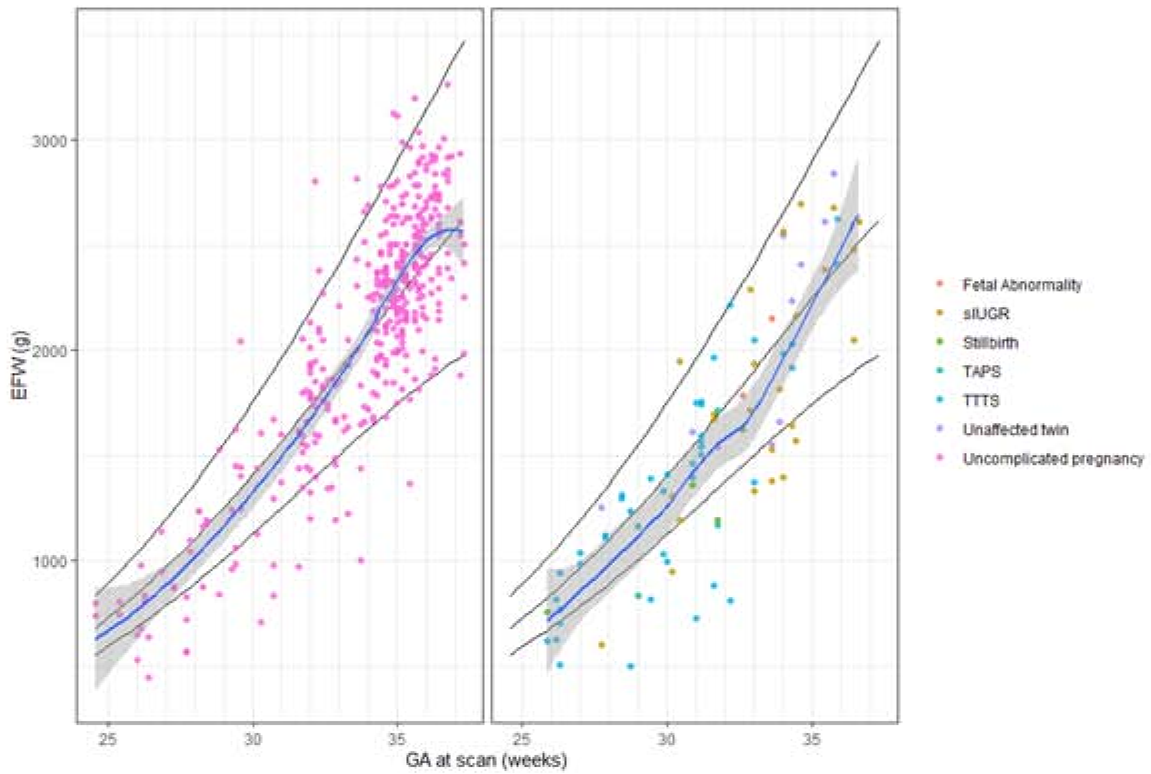
35
GA at delivery (weeks)
Fetal Abnormality
sIUGR
Stillbirth
TAPS
TTTS
Unaffected twin
Uncomplicated pregnancy

Figure 6



3000
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EFW (g)
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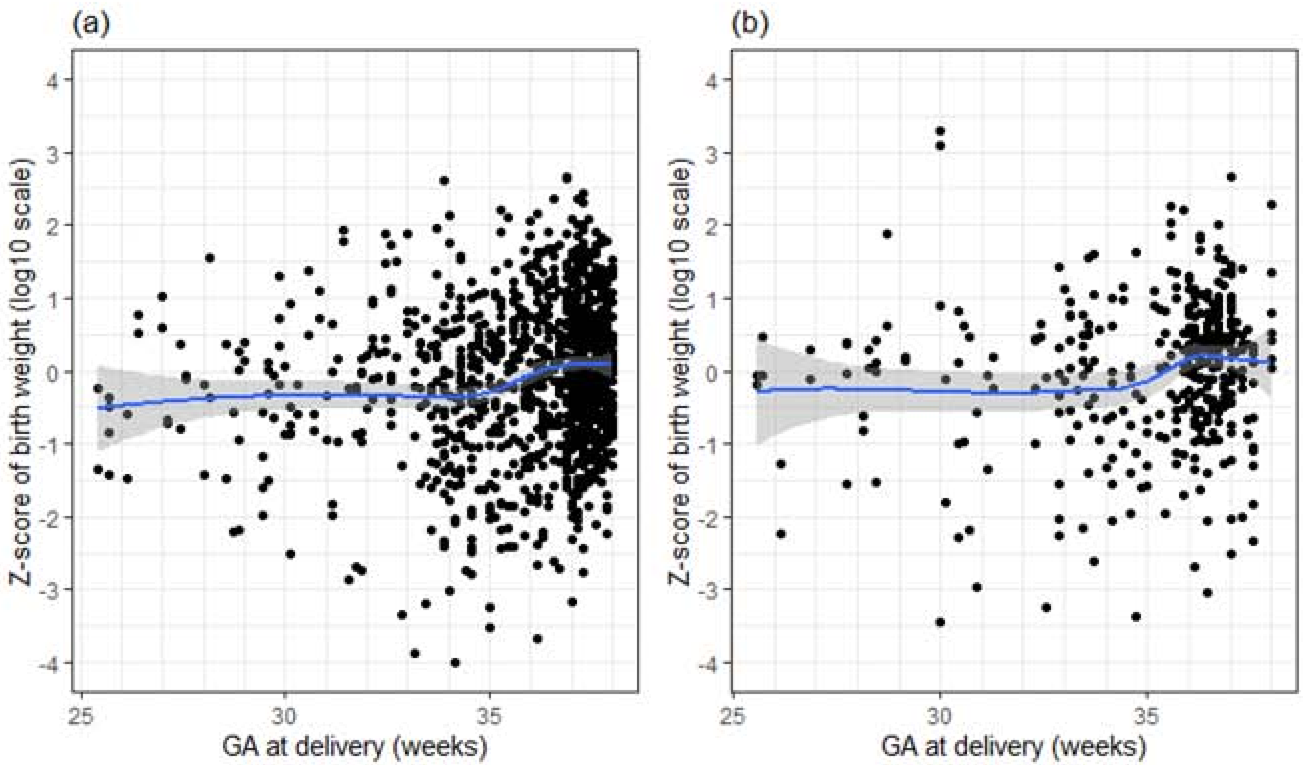
GA at scan (weeks)
Fetal Abnormality
sIUGR
Stillbirth
TAPS
TTTS
Unaffected twin
Uncomplicated pregnancy

Figure 7



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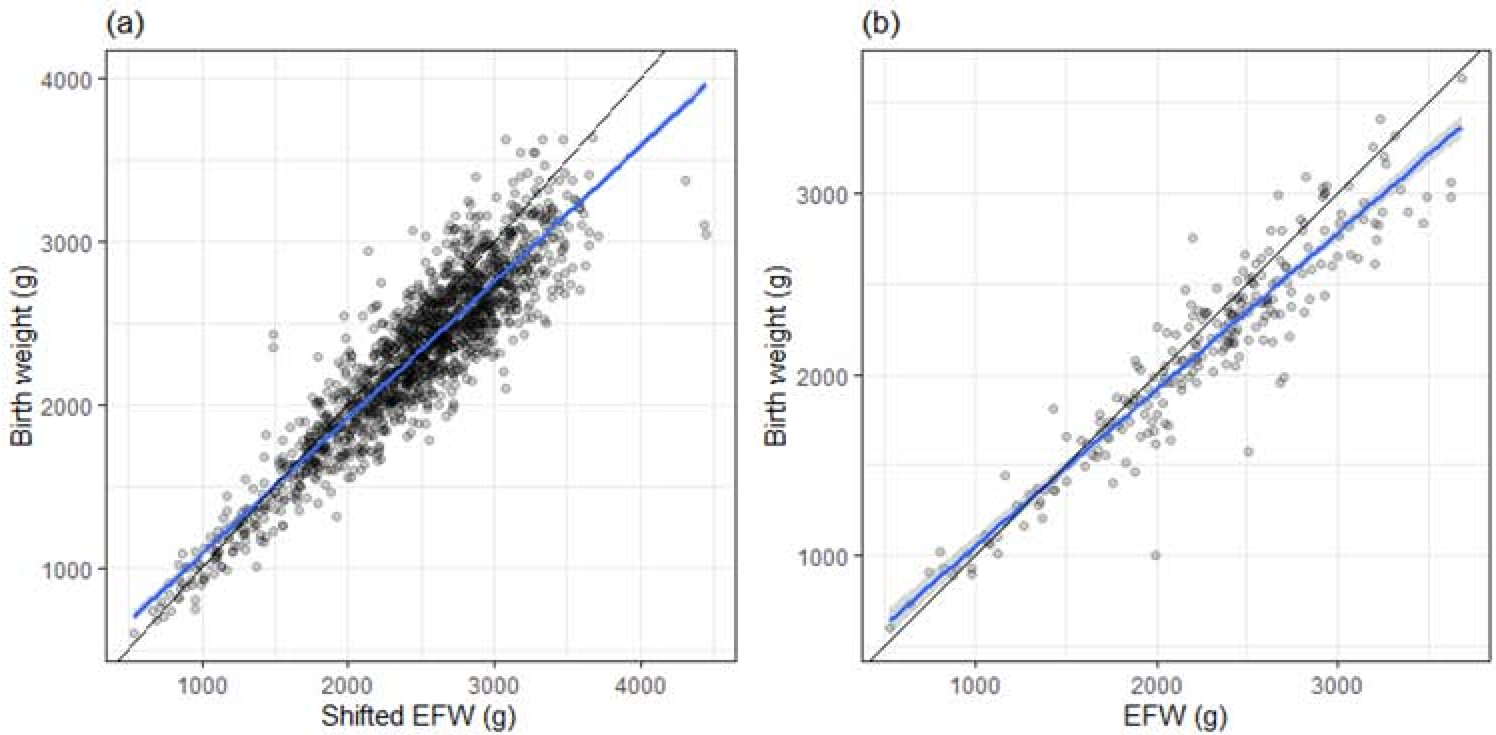

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