


Research Article

Fetal Growth Chart in Twins Consistent with The FMF Growth Curve: Prospective Multicenter Study

Hamutal Meiri, PhD^{1*}, Nadav Kugler, MD¹, Nataly Sharon, MD¹, Ran Svirsky, MD^{1,2}, Richard Brown, MD³, Heidy Portillo Rodriguez, MD³, Anna Gonc , MD, PhD⁴, Mar Bennasar, MD, PhD⁴, Antoni Borrell, MD, PhD⁴, Julia Ponce, MD⁴, Annegret Geipel, MD, PhD⁵, Adeline Walter, MD⁵, Corinna Simonini, MD⁵, Brigitta Strizek, MD, PhD⁵, Elisa Bevilacqua, MD, PhD⁶, Federica Romanzi, MD⁶, Karl Oliver Kagan, MD, PhD⁷, Tanja Lennartz, MSc⁷, Armin Bauer, PhD⁷, Ron Maymon, MD¹, Kypros H. Nicolaides, MD⁸, Yoram Louzon, PhD⁹ and the Pre-Twin Screen Consortium¹⁰

Abstract

Objectives: Different retrospective growth charts for twin pregnancies debates whether twin growth is similar to singletons. Recently, the fetal medicine foundation (FMF) published a retrospective fetal growth chart that indicated similar growth charts for twins and singleton if only term delivery of both twins without major malformation are included. A national Danish growth chart confirmed these findings.

We constructed prospective growth chart of estimated fetal weight (EFW) throughout pregnancy of monochorionic diamniotic (MCDA) and dichorionic (DC) twins using a multi-center, multi-national cohort.

Study Design: Pregnancies with two live fetuses at 11-13 wks' gestation were enrolled. Gestational age was determined from crown-rump length of the larger twin in the first trimester. EFW charts were made from fetal growth at 11-13, 20-22, 24-26, 28-30, 32-34, and 36-37 wks' gestation using Hadlock-4 formula made of biparietal diameter, head and abdominal circumference, and femur length. Chorionicity specific charts were built for 376 DC and 158 MCDA live twins who were born at term, without malformations. Centers included were from Montreal, Canada, Bonn and Tübingen, Germany, Barcelona, Spain, Rome, Italy, and Zefin, Israel. ANOVA was used to compare ethnic and centers charts and functional fit to were compared our charts to retrospective EFW charts of the FMF.

Results: Growth was slower in MCDA compared with DC twins starting from 21st wks' gestation and increased with advancing gestation. Our charts were similar but marginally slower than the FMF ones. Smaller EFW were in Barcelona and Rome compared to the larger in Bonn and Tübingen, and fetuses of white women were larger than those of other ethnicities

Conclusions: We developed a prospective growth chart for pregnancies with two live DC and MCDA twins born at term without malformations that are consistent with those of the FMF, with small differences between countries and ethnicities.

Affiliation:

¹Department of Obstetrics and Gynecology, Shamir (Assaf Harofeh) Medical Center, Zefin, and Faculty of Medicine and Health Sciences, Tel Aviv University, Israel.

²Medical Genetic Unit, Department of Obstetrics and Gynecology, Samson Assuta Ashdod University Hospital, Ashdod, Israel, and School of Medicine, Faculty of Medicine and Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er Sheva, Israel

³Division of Maternal Fetal Medicine, Department of Obstetrics & Gynaecology, McGill University, McGill University Health Centre, Montreal, Canada

⁴Fetal-Maternal Medicine Department. BCNatal., Hospital Clinic, University of Barcelona. Sabino Arana 1, 08028 Barcelona, Catalonia, Spain

⁵Department of Obstetrics and Prenatal Medicine, University Hospital Bonn, Bonn, Germany

⁶Department of Women and Child Health, Women Health Area, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

⁷Maternal Fetal Medicine Unit, Department of Obstetrics and Gynecology, University Hosp Tübingen, Tübingen, Germany

⁸The Fetal Medicine Research Center, King's College Hospital, London, UK

⁹Department of Mathematics, Bar Ilan University, Ram Gan, Israel

¹⁰The Pre-Twin Screen Consortium, Tel Aviv, Israel

*Corresponding author:

Dr. Hamutal Meiri, Pre-Twin Screen Consortium, 24 Beit El St. Tel Aviv 6908742, Tel Aviv, Israel.

Yoram Louzon, Department of Mathematics, Bar Ilan University, Ram Gan, Israel

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Introduction

The proportion of twin pregnancies has grown over the last 20 years, from 1.5–2.0% to 2.5–3.0% of all pregnancies, due to increased rates of conception using assisted reproduction technology and increasing maternal age at conception [1–3]. In twin compared with singleton pregnancies, there is a substantially higher incidence of adverse pregnancy outcomes, including higher rates of preterm birth, preeclampsia, fetal growth restriction, gestational diabetes mellitus and perinatal death [3–8]. Moreover, the overall rate of fetal malformations, as well as the incidence of several specific fetal defects, is higher in twin pregnancies (whether same or different gender) than in singleton pregnancies [9]. Consequently, the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) and various national societies recommend increased surveillance of twin pregnancies including 5–6 antenatal ultrasound scans for dichorionic (DC) twin and 7–8 for monochorionic diamniotic (MCDA) twin pregnancies [10–14].

This study is a part of the 2019's Era PerMed Fund of the European Commission and national health research authorities – project 'Pre-Twin Screen' (trial #JCT2019-61). This project aims to establish a personalized international model for prenatal fetal monitoring that can be used to develop a 'gold standard' for the antenatal care of twins [15], to cover the gap of a relatively limited number of multinational and multicenter studies regarding twin pregnancies.

The objective of this study was to generate prospective, multicenter, multinational derived reference charts for estimated fetal weight (EFW) with advancing gestational age in pregnancy of two live twins born at term without major structural or genetic malformations, assuming that this study nature could represent global growth trajectory in twins.

Recently, the Fetal Medicine Foundation (FMF) published twin growth charts that indicated similar growth charts for singleton and twins when term delivery of two live twins without malformations are included [16]. Validation of these charts for DC and MCDA twins for fetal growth in twins was subsequently obtained from a nationwide Danish cohort study [17]. Some similarities were also included in a prospective study from Ireland [18]. Thus, another objective of our study was to compare our charts to the ones of the FMF, Denmark and Ireland charts for fetal growth in twins.

Methods

Study population

This study is a part of the ERA PerMed funded Pre-Twin Screen project on the evolution of twin pregnancies (JTC2019-61). Ethics approval was obtained from Shamir Medical Center, Zefirin, Israel (Trial #: 0043-20-ASF, Israel Ministry

of Health Authorization #202016632), and subsequently from all other participating centers. All participants provided written informed consent. The protocol was registered at ClinicalTrials.gov (ID #: NCT04595214).

Enrolment to the study started in December 2020 and ended in August 2023; the last delivery of the included pregnancies was in February 2024. We prospectively enrolled women with two live MCDA and DC fetuses at 11+0 to 13+6 wks' gestation, dated using the crown–rump length (CRL) of the larger twin [19]. The study enrolled 649 women with a twin pregnancy (1298 fetuses at enrolment).

For the purpose of developing the growth chart of the DC and MCDA twins we excluded 86 women (21 who lost both twins, 23 who lost one twin, 6 who had twin reduction to singleton due to major malformations, and 36 who relocated abroad), leaving 563 women and 1126 live twins at birth. In addition, we excluded all DC twins delivered before 37 week's gestation and MCDA twins delivered before 36 wks' gestation leaving us with a total of 534 twin fetuses – 376 DC and 158 MCDA fetuses from pregnancies of two live twin delivered at term without malformation.

Pregnancy assessment

First trimester (11–13 wks' gestation) cell-free DNA was tested to identify the major fetal trisomies (21, 18 and 13) was carried out at the enrollment week visit. In cases of identified trisomy, genetic counselling of the parents followed by invasive chorionic villous sampling (CVS) or amniocentesis followed by chromosomal microarray (CMA) or whole-exome sequencing (WES) were offered, at parental choice. In DC twins, when trisomy was confirmed in one fetus, further parental counseling was provided, including the option of selectively reducing the affected twin. Cases of fetal reduction or spontaneous death of one twin remained in the study and were followed-up until delivery but were not included in the current analysis.

In the first, second and third trimesters thorough ultrasound examinations were carried out for complete fetal anatomical survey, and major malformations were identified. Parental consultation was conducted, with parental decision for reducing one (DC twin) or whole pregnancy termination (TOP) of MCDA twins or continuation with the pregnancy. Cases of fetal reduction or spontaneous death of one twin remained and cases with fetal malformations that continue with the pregnancy remained in the study and were followed-up until delivery but were not included in the current analysis.

To determine the EFW according to Hadlock et al. [20] the 4 formula measurements for biparietal diameter (BD), head (HC) and abdominal (AC) circumference, and femur length (FL) were measured. fetal biometry was measured at 11–13, 20–22, 24–26, 28–30, 32–34 and 36–37 wks' gestation,

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unless the pregnancy had been delivered earlier. In MCDA twin pregnancies additional ultrasound scans were carried out at 15-16 and 17-18 wks' gestation (not displayed in the results curves hereunder).

All women were offered screening for pre-eclampsia at 11-13 wks' gestation using a combination of maternal risk factors, mean arterial pressure, uterine artery pulsatility index and maternal serum placental growth factor [23]. Those identified as being at high risk for pre-eclampsia (risk cut-off 1/100) were treated with aspirin (150 mg/day until 36 wks' gestation) [23]. Of note, in Barcelona all included women had already started treatment with 150 mg/day of aspirin by the time of study recruitment as per local guidelines.

Delivery records were collected from the hospital delivery units, or if delivered elsewhere, data were obtained from the discharge reports after delivery and by interviewing the mother.

Diagnosis of gestational diabetes mellitus (GDM) was determined at 24-28 wks' gestation according to the American College of Obstetrics and Gynecology (ACOG) guidelines [24] with variations in Barcelona according to local guidelines [25] Women with GDM were treated by diet, metformin and/or insulin, as necessary.

Cervical length was measured by transvaginal or transabdominal ultrasound at 20-24 wks' gestation as a part of the second trimester anatomical evaluation or at 24-28 wks' gestation. When cervical length < 25 mm identified, vaginal progesterone was used [26].

Women who attended the labor ward with suspected preterm delivery were treated with corticosteroids and/or additional drugs, according to local guidelines [27].

Statistical analysis

The primary purpose of the analysis was to model the growth in twin pregnancies, as measured by EFW. Analysis included only the cases with two live births, born at $\geq 37+0$ wks' gestation in DC twins or $\geq 36+0$ weeks in MCDA twins. In each pregnancy, EFW measurements were taken at six different timepoints: 11-13, 20-22, 24-26, 28-30, 32-34 and 36-37-wks' gestation. We compared the EFW between subgroups, categorized by either chorionicity, ethnicity or center.

The differences in EFW between ethnic groups and centers were compared using ANOVA, with time point as a discrete independent variable on the measurement and ethnic groups or center, and ANOVA on each time point by itself, as well as multiway ANOVA including all factors. We developed a functional fit model for EFW time dependence and performed nonlinear mixed-effects modelling to compare our results

with those of the FMF. For each set of EFW measurements, ANOVA was performed to assess the difference between groups [28]. If only two groups were defined, the ANOVA was replaced by a two-population t-test. Results are presented as uncorrected P-values. To compare the observed EFW with published FMF data, we performed two-population t-test. However, since the variance of the FMF data is not reported, we estimated the variance around the EFW median value using the difference between the 10th and 90th percentile in the FMF data, assuming the values around the median centile has a normal distribution. The comparison was also performed using NLME analysis [29] with both the coefficient and the power evaluated according to fixed and random effects.

To compare data from different centers and ethnic groups within our study, we performed ANOVA to compare the growth curves, using timepoint (week of measurement) as a discrete independent variable. We also analyzed EFW for each center and for ethnic groups across centers, using EFW as the independent variable. When comparing our results to the FMF, the analysis was performed separately for DC and MCDA twins.

To compare twins within the same pregnancy, the EFW of each twin was translated to its relative rank (between 0 and 1) at the appropriate time point. We then computed the Spearman correlation at different time points between pairs of twins within the same pregnancy. We further computed the Spearman autocorrelation between EFW rank in consecutive weeks for the same twin and performed a linear regression on the rank using the two previous time points.

Additionally, a function of $(a + a_i) * t^{\gamma + \gamma_i}$ was fitted to EFW at time t , where a and γ represent the fixed effects and the addition of the index i represents random variation. The model was fitted using nonlinear mixed-effects modelling (NLME) for our samples. For the FMF data, we used a linear regression of the log EFW on the log time. We compared the variance of the random effect for each variable, and the fixed effect in our data vs the FMF regression results [28].

In addition to the above, we evaluated the power of EFW analysis of twin curve results from the same mother. To compare these growth curves, we fixed the coefficient for all mothers (i.e. only allowed a fixed effect) and computed the power of EFW as a function of the time point. We then compared this power between twins from the same mother.

Continuous variables were presented as median (interquartile range (IQR)) and the differences between groups were compared using the Mann-Whitney U-test or Kruskal-Wallis non-parametric test. Categorical values were presented as n (%) and were compared using the Chi-square test or Fisher's exact test. All estimates and statistical tests were performed using MATLAB version 2023b (MathWorks

Inc., Natick, MA, USA; https://www.mathworks.com/products/new_products/release2023b.html). Power analysis was conducted using WinPepi software (version 11.65 www.brixtonhealth.com/pepi4windows.html).

Results

Study population

Among the 267 women included in the analysis (534 fetuses) the enrolment was at a median gestation week 12.6 (IQR, 12.1-13.0), at a median age of 34.8 (IQR, 31.2-37.6) years and a median body mass index of 24.5 (IQR, 21.7-28.0) kg/m². The majority of the women (87.0%) were of white ethnicity; non-white ethnicities were black, Hispanic and

Asian. Overall, 57.7% of the women were nulliparous and the pregnancy was conceived spontaneously in 58.4% of cases, which increased to 70.9% among MCDA pregnancies. The median gestational age at delivery was 37.4 (IQR, 37.1-37.9) weeks for DC pregnancies and 36.6 (IQR, 36.3-37.1) weeks for MCDA pregnancies.

Among the 534 fetuses, the median birth weight was 2647 (IQR, 2420-2894) g for DC pregnancies and 2430 (IQR, 2258-2659) g for MCDA pregnancies. Female fetuses comprised 46.8% of the total study cohort, with a median birth weight of 2620 (IQR 2420-2870) g for female neonates in DC twins and 2370 (IQR 2236-2595) g for female neonates in MCDA twins.

Table 1: Characteristics of 551 dichorionic (DC) or monochorionic diamniotic (MCDA) fetuses in twin pregnancies included in the study.

Maternal characteristics				
Features	All mothers (n=267)	DC pregnancies (n=188)	MCDA pregnancies (n=76)	P*
Maternal age (years)	34.8 (31.2-37.6)	34.8 (31.4-37.6)	34.7 (30.4-37.1)	0.34
GA at enrolment (weeks)	12.7 (12.3-13.0)	12.7 (12.2-13.0)	12.6 (12.3-13.0)	0.82
BMI (kg/m ²)	24.5 (21.7-28.0)	24.8 (22.0-28.7)	23.3 (21.3-27.5)	0.01
Ethnicity n (%)				
White	232 (87)	164 (62)	68 (25)	0.67
Non-white	35 (13)	24(9)	11(4)	
Parity n (%)				
Nulliparous	154(58)	105 (39)	46(19)	0.550
Parous	113(42)	83(31)	30(11)	
Conception method n (%)				
Spontaneous	156(58)	105(37)	56(21)	
In-vitro fertilization	91(34)	70(26)	21(8)	0.017
Ovulation induction	40(8)	36(7)	4(1)	
GA at delivery (weeks)	37.3 (37.0-37.7)	37.4 (37.1-37.9)	36.6 (36.3-37.1)	0.54
Neonatal Characteristics				
Features	All fetuses (n = 534)	DC (n = 376)	MCDA (n = 158)	P*
Female n (%)	250 (47)	178 (47)	72 (46)	< 0.001
Birth weight (g)	2579(2355-2827)	2647(2420-2890)	2430(2258-2659)	< 0.001
Female (g)	2550(2330-2766)	2620(2420-2870)	2370(2236-2595)	< 0.001
Male (g)	2600(2380-2880)	2670(2423-2961)	2485(2295-2745)	< 0.001

Data presented as Median (interquartile range) or n (%). *Comparison of DC and MCDA twins.

BMI, body mass index; GA, gestational age.

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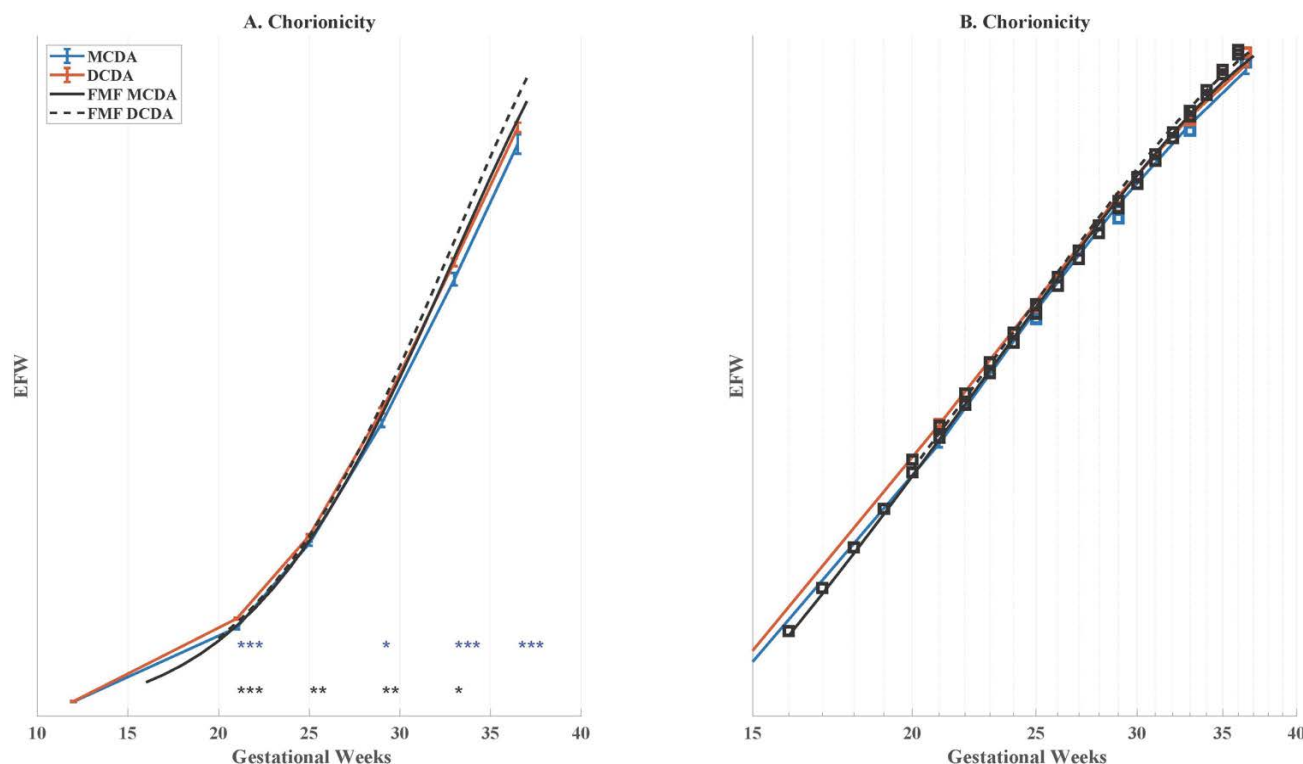


Figure 1: Estimated fetal weight (EFW) according to chorionicity.

A) Growth curves of monochorionic-diamniotic (MCDA) (blue line) and dichorionic (DC) (red line) twins in the Pre-Twin Screen study compared to dashed line (MCDA) and black line (DC) twins in the Fetal Medicine Foundation (FMF) study [16]. Points – medians, curves fitted to the model (respective color). FMF data - 20-22; 24-26; 28-30; 32-34; 35-36 and 37-38 wks' gestation. Pre-Twin Screen data - 11-13, 20-22, 24-26, 28-30, 32-34 and 36-37 weeks. Difference between groups by ANOVA, asterisks - * for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$ (black for comparing DC to MCDA twin in Pre-Twin Screen study, blue for comparing DC and MCDA twins between Pre-Twin Screen and FMF studies).

B) Log-log linear power law regression fits. Color code as in A. Curve's order top to bottom: FMF DC – $EFW=0.011*week^{3.48}$ and MCDA twins $EFW=0.008*week^{3.56}$ Pre-Twin Screen DC – $EFW=0.01*week^{3.37}$ and MCDA twins – $EFW=0.01*week^{3.39}$.

Table 2: Estimated fetal weight (g) according to gestational week (GW), stratified by chorionicity, ethnicity and center.

Gestational week	Centile				
	5 th	10 th	50 th	90 th	95 th
Chorionicity					
MCDA fetuses (n=158)					
Nov-13	48.5	52	62	77.8	82.3
20-22	284.1	301.4	371	507.2	523.8
24-26	596.7	625.6	749	898.6	932.7
28-30	1029.3	1059.7	1242	1545.2	1637.6
32-34	1560.2	1648.5	1931	2323.9	2375.4
36-37	2110.2	2192.2	2543	2803.8	2891.4
DC Fetuses (n=376)					
Nov-13	50	53	65	80	86
20-22	326.2	343	423	533	562
24-26	632.4	659.6	788	967.3	1012.5

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28-30	1056.4	1107.8	1335	1604.4	1691.3
32-34	1572.8	1644.8	2011	2380	2474.3
36-37	2033.7	2151	2599	3021	3175.7
Ethnicity					
White (n=464)					
Nov-13	50	52.3	64	80	86
20-22	312	331.6	414	524.4	559.2
24-26	625.4	655.2	781	948	1003
28-30	1054.2	1105.4	1319.5	1595	1664
32-34	1604.3	1694	1980	2350.4	2451.2
36-37	2077	2167	2607	3003.4	3177.5
Non-white (n=70)					
Nov-13	46.45	53.2	63	80.2	85.55
20-22	298.85	310.8	383	507.2	529
24-26	566.2	614.2	746	973.8	1026.4
28-30	1003.5	1057	1283	1655	1687.7
32-34	1494.2	1547.1	1867.5	2398.9	2441
36-37	2014	2063.6	2442.5	2917.3	2984
Centers					
Barcelona, Spain (n=116)					
Nov-13	45.25	55	67	81	88
20-22	315.2	325	374	472	512
24-26	629.4	641.9	742	891.4	980
28-30	1061	1087	1283.5	1494.2	1672.8
32-34	1607.6	1644.9	1898	2412.9	2507.4
36-37	1915.5	1998	2492.5	2748	2811
Rome, Italy (n=76)					
Nov-13	51.3	53.1	60	70	72.7
20-22	285.3	303.7	375.5	470.2	478.7
24-26	602.4	622.3	725	885	930.8
28-30	1037.1	1072.4	1253.5	1514.5	1563.7
32-34	1639.4	1713.8	1940	2227.3	2274.8
36-37	2102.8	2206.5	2623	2988.7	3075.6
Montreal, Canada (n=70)					
Nov-13	50.7	52	60	71	71.5
20-22	303	315	374	500.5	508
24-26	596.7	613	706.5	921.8	978.4
28-30	965	1025.7	1206.5	1589.8	1748.4
32-34	1435	1516	1873.5	2247	2354
36-37	2029.7	2158.1	2575	2919.6	3087.2
Zerifin, Israel (n=78)					
Nov-13	46	46.3	58	77.7	86.4
20-22	409.6	432	510	610.3	645
24-26	580	671.6	896.5	1036.3	1049.4
28-30	1036.3	1063.6	1291	1525	1569.1

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32-34	1534.5	1585	1926	2263.4	2333.4
36-37	1997.6	2074.4	2536	2977.8	3093.3
Tubingen, Germany (n=64)					
Nov-13	49.65	51.8	65	88.2	89
20-22	299	312	386	503.2	593.8
24-26	631.2	670	791	975.8	1017.8
28-30	1101.9	1144.4	1374.5	1673.4	1836.6
32-34	1641.5	1767.5	1997	2425.5	2460.5
36-37	2014.6	2121.4	2509	2994	3152.6
Bonn, Germany (n=130)					
Nov-13	57	58.5	68	79	83
20-22	349.6	363.2	429.5	510.5	539.8
24-26	660	708.5	818	946	1007
28-30	1135.5	1214.2	1425	1656.4	1704.1
32-34	1733.7	1836.2	2089.5	2413.4	2565.5
36-37	2138.8	2194.2	2661.5	3115.2	3255.8

Data are given as medians. 3rd centile not shown as it represents 10 or less cases. DC, dichorionic; MCDA, monochorionic diamniotic.

Growth curves The growth of MCDA and DC twin was not significantly different from each other until 21 wks' gestation, from which the growth of MCDA twins was significantly slower. The differences increased with advancing gestation (Figure 1a, Table 2). There were small differences in growth between white and non-white groups. The EFW of fetuses in white women (2607 g) being larger than those of other ethnicities (2442 g, forming 165 g difference at 50th centile at 36-37 wks' gestation) (Figure 2a, Table 2). Among the participating centers, the smallest fetuses were from Barcelona and Rome and the largest were from Bonn and Tubingen; those from Israel and Montreal were in the middle (Figure 2B, Table 2). The maximum difference between Bonn and Barcelona was 77 g for the 50th centiles at 36-37 wks' gestation. Comparison between female and male fetuses was non-significant.

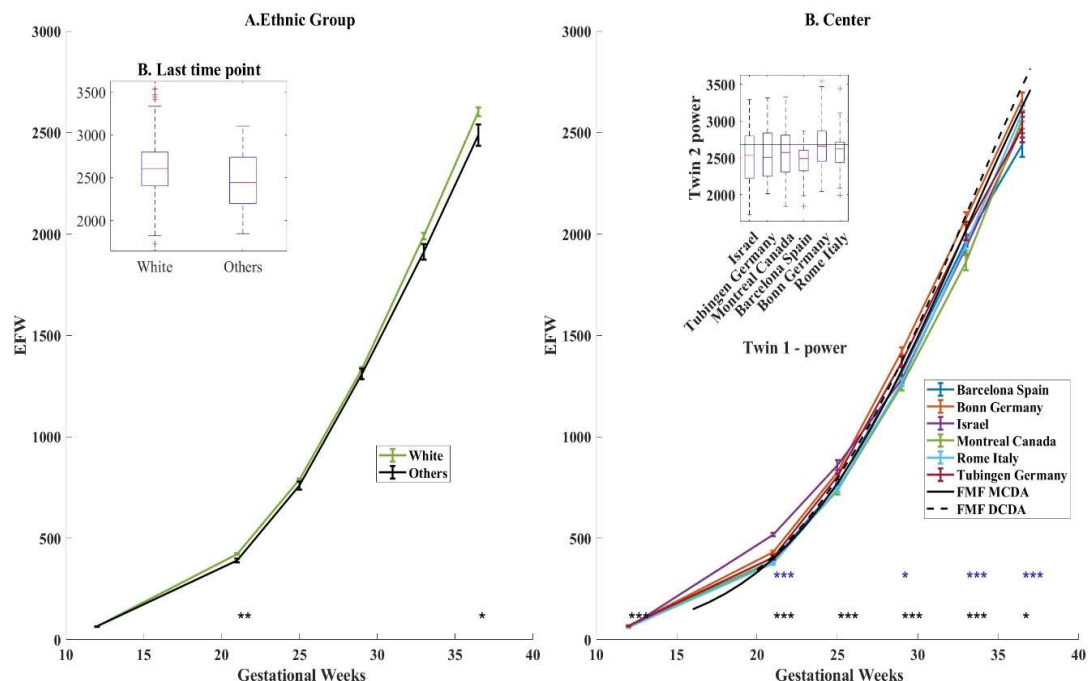


Figure 2: Estimated fetal weight (EFW) by ethnicity and centers.

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A) Ethnicity: The EFW of the white (Green dots and lines) compared to non-white cases as measured for 11-13, 20-22, 24-26, 28-30, 32-34, and 35-36 wks' gestation with ANOVA to calculate the difference between groups. Black asterisk - $p < 0.05$.

B) Centers: The EFW compared by centers and to FMF curves. Order- smallest to largest: Barcelona (blue), Rome (green), Montreal (purple), Israel (yellow), Tubingen (Turquoise), and Bonn (red). The latter two are FMF (black and dashed line). Note - Tubingen and Bonn are the closest to FMF, consistent with similar ethnicity. The significance of the differences between groups (ANOVA) is marked with black asterisks, and between this and FMF study in blue asterisks as in Figure 1.

C, D) Box plots Comparison of the EFW in 36-37 wks' gestation is shown for ethnic groups (C) and for centers (D). Color code – C as in A and D as in B.

The patterns of growth for both DC and MCDA twins were similar at the end of the first trimester (11–13 weeks), but from 21 wks' gestation they start to differ. The median of the EFW curve at 24-26 wks' gestation was 788 g in DC twins versus 749 g in MCDA twins, and subsequently 1335 g vs 1242 g at 28-30 wks' gestation, 2011 g vs 1931 g at 32-34 wks' gestation, and 2599 g vs 2543 g at 36-37 wks' gestation in DC vs MCDA twins, respectively.

The EFW of twins from all our centers of Pre-Twin Screen study was on average smaller than that in the FMF cohort, but

the difference was smaller when comparing the FMF cohort (Figures 1 and 3) to the results from Bonn and Tubingen (Figures 2 b), and similar to the differences between fetuses in the two German centers compared to those in Rome and Barcelona (Figure 2 b). As shown in Figure 1B, our chart for the entire study provides a good statistical fit to the FMF data. The minor differences between the growth charts of the entire Pre-Twin Screen Study and the FMF growth charts (Figure 1 and 3) are similar to the differences in our Pre-Twin Screen study charts of fetuses from Rome vs those from Bonn (Figure 2). Consequently, the differences, either for all twins or for each of the DC and MCDA twins are likely related to the different ethnic and population compositions between our whole Pre-Twin Screen and the ethnic and population composition used to build the FMF chart. EFW according to Pre-Twin Screen can be calculated at <https://twin-fwe.math.biu.ac.il/>.

The best fit to a growth curve was obtained by fitting the EFW to a power law (Figure 1b). The fit was excellent until almost the end of the pregnancy, with a very similar fit for our DCDA and MCDA in the present study to their respective chorionicity groups from the FMF study. The power was within the narrow range of 3.37-3.56 and the constant was between 0.008 and 0.011.

Using the NLME analysis [29], the variance of the

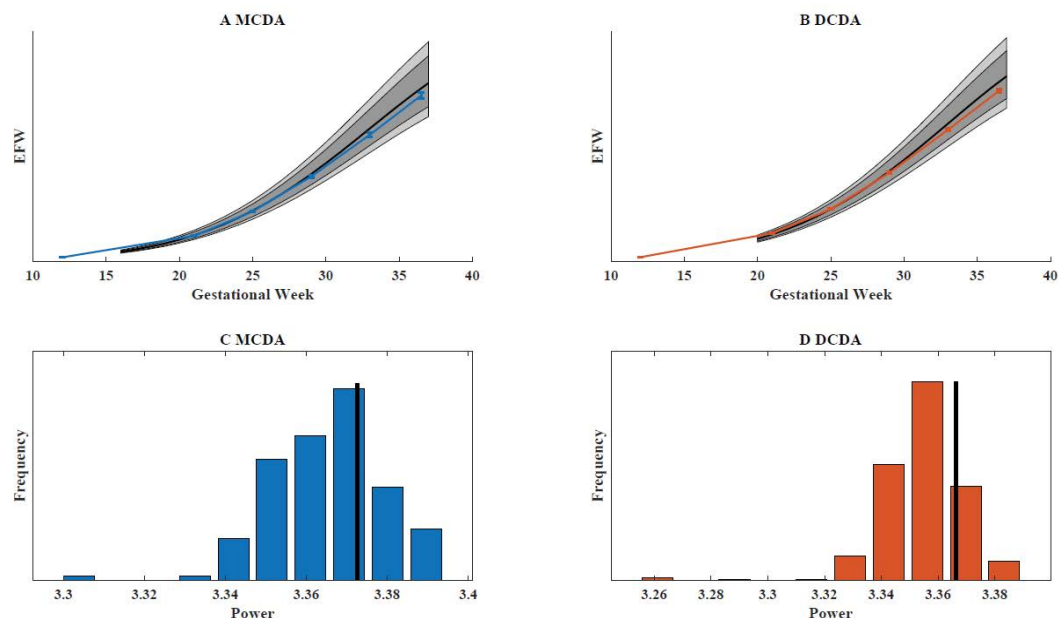


Figure 3: EFW of monochorionic diamniotic (MCDA, left) and dichorionic (DC, right) twins.

A, B - Medians (dark lines) of the FMF curve compared to Pre-Twin Screen (red) curve for DC (A) and MCDA (B) twins. Shaded areas – gray: 10th to 90th centiles, light gray - the 5th and 95th centiles.

C, D - Histograms of power of the fit to the power law (proportional to the week at a given power between 3.25 and 3.4) for individuals in MCDA (C) and DC (D) twins.

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coefficients calculated according to fixed and random effects were negligible compared to the power ($\text{Var} < 1 \times 10^{-10}$ for coefficient, compared with 1×10^{-3} for power). We thus replaced the model to have a random effect on only the power (Figure 1b). We then fixed the coefficient for the parallel curve of the FMF chart and fitted the power with a linear regression (Figure 1b). The difference between the coefficients of the powers of the fit in our data vs the parallel FMF data was not significant.

Given the possible interaction between the different terms, we performed multiway ANOVA separately for the centers ($P < 1 \times 10^{-10}$) or for the ethnicity group ($P < 1 \times 10^{-10}$), for timepoint ($P < 1 \times 10^{-10}$) and for chorionicity ($p < 0.001$).

Figure 3 compares the FMF growth chart with the Pre-Twin Screen curve. In essence it shows that our prospective, multisite and international study confirms the retrospective study of the FMF in either the longitudinal chart or the histogram for each of DC and MCDA twins' charts. These finding are important for identifying fetuses of restricted

growth that needs special attention. As we did not have the FMF cohort database, we verified the similarity of our findings to FMF chart by performing a linear transformation to fit each point to the 10-90th range. It was done by removing from each value the average of the FMF data, and divided the remaining value by the FMF 10th or 90th percentile difference and added 0.5, so that if the average would be the same. The black line in bottom of Figure 3 for DC and MCDA twins of both the upper and lower part of the figure marks the mean (the 0.5 point). Accordingly, when the average (vertical line in the histogram and longitudinal line in the chart) would be at the FMF's 10th or 90th percentile, the line crosses the horizontal axis at the value of 0.1.

In addition to the above, we compared the growth curve of twins from the same mother. using by computing the correlation coefficient as described in the methods. In this way we obtained a high (0.74) correlation coefficient between twins of the same mother, regardless of some discordance which may occur among twins (Figure 4).

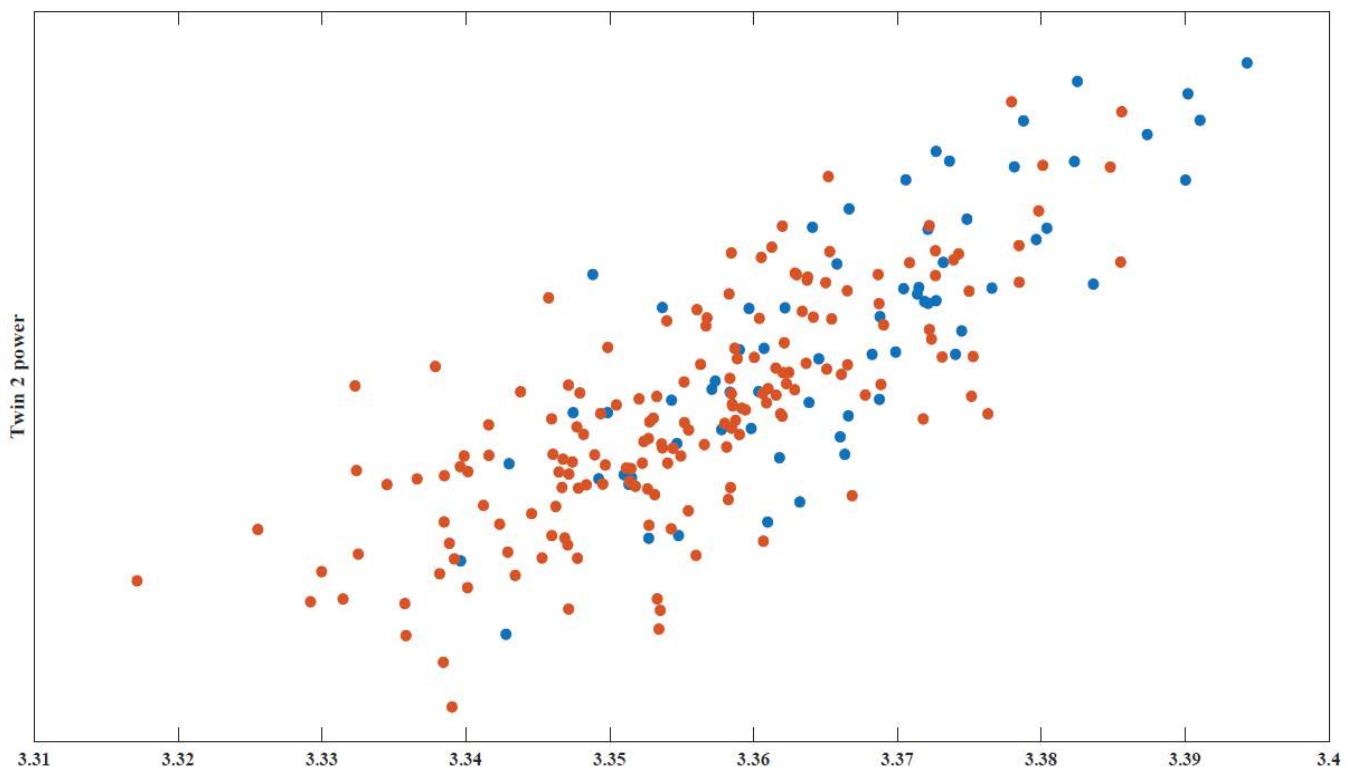


Figure 4: Comparison of twins from the same mother.

Slope of the fit for pairs of twins from the same mother (the power of the EFW as a function of the gestational week). Powers have high fit to the correlation coefficients (CC)= 0.74 and 0.83 for DC and MCDA twins, respectively ($p < 0.001$ for either one). Red and blue dots - DC and MCDA twins, respectively.

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Discussion

Main findings

This is the first prospective, multicenter, multinational study using EFW to evaluate the growth trajectory in DC and MCDA twin pregnancies with two normal live neonates delivered at $\geq 37 + 0$ weeks and $\geq 36 + 0$ wks' gestation, respectively. Interestingly, this normal growth curve, for both DC and MCDA twins, is similar to that reported by the FMF for a retrospective analysis, and the nation-wide Danish study [16,17]. Minor differences are seen in the variation of growth trajectories between centers and ethnic groups within our study. Growth curves of DC and MCDA twins are indistinguishable until week 21 and from there, a slower growth is depicted for MCDA twins.

Comparison with findings of previous studies and clinical implications

Our study focused on creating growth trajectory charts for normal twins who were liveborn at term, after exclusion of those with early delivery, malformations or spontaneous or iatrogenic death of one twin. These growth trajectories enable the description of normal growth patterns in twins that can be compared to those of singletons, as in the FMF study [16].

The FMF study [16] compared the pattern of growth in nine previously published models and reported that growth in twins, relative to the FMF singleton growth charts, was similar in three of these studies. All models showed a period of deceleration in growth, relative to the FMF reference, in the early third trimester. However, in contrast to the FMF model, five of these studies demonstrated catch-up growth in the second half of the third trimester [16]. The FMF chart is unique as it is the first to present an innovative view that the normal twin growth chart is similar to the singleton growth chart. Thus, where smaller twin fetuses were considered normal previously, the FMF chart considers them as growth-restricted and enables fetal medicine specialists to focus their attention on such twins and provide them with the careful surveillance that is needed. Accordingly, our independent prospective, multicenter, multinational analysis is important in showing similarity with the FMF curve, as opposed to other formerly published curves [30-38].

The FMF chart for twins [16] was also supported by a 10-year nationwide Danish cohort study[17], although they used the Hadlock-3 parameters formula and not the 4-parameter one that we used to calculate the twin EFW chart. Both the FMF and the Danish studies included only two live twins delivered at > 37 wks' gestation in DC and > 36 weeks in MCDA pregnancies, as we did here compared to the inclusion of twin delivered preterm and twins with major malformations as was included in the other charts [30-38].

The FMF study included data from the second trimester and used three biometric measures to calculate the EFW. In the present study, we included data from the first trimester and calculated the EFW from four biometric measures. The adoption of the growth models from this study and of the FMF Chart for twin pregnancies enables the prospective identification of fetuses with growth trajectory patterns falling outside of the depicted standard, which should be used for assessment of growth. It should be noted that these charts showed growth curve similarities in singleton and twin pregnancies.

Strength and Limitations

One may argue that Pre-Twin Screen study has a relatively small number of twin pregnancies included in the analysis after excluding cases as listed before. Power calculation for detecting ethnicity differences generated a power=0.95 with an alpha=0.05, requiring a sample size of 220 cases, while our study included more than twice that number of cases. Regarding the centers, it seems that there is no need for the simulations to show that the sample size is large enough to identify significant statistical differences between centers, that are much larger than the standard error within each group

The main strengths of this study are the multicenter and multinational nature of the prospective data collection, conducted by a multidisciplinary team of experts, and the rigorous exclusion of all abnormal cases. Consequently, our results are generalizable. We obtained similar results to the charts reported by the FMF,16 which were derived from the retrospective analysis of a larger population. In addition, we generated curve-fitting equations for EFW, which have not been published previously and made comparisons between growth curves generated using NLME and regression analysis.

Conclusions

In this prospective, multicenter and multiethnic study of twin pregnancies, fetal growth trajectories in DC and MCDA twins were consistent with those reported by the FMF [16]. The trajectories demonstrate progressive differences in the rate of growth between DC and MCDA twins after 24-26 wks' gestation, with small differences in growth patterns also being noted between different countries and ethnicities.

Key Points

- First multi-center, multi-national prospective fetal growth chart in twins (66)
- Growth pattern varies among ethnicities and countries (47)

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- The growth chart validates of the FMF ones for mono and di-chorionic twins (63)

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Ethics

Ethics approval was obtained from Shamir Medical Center, Zerifin, Israel (Trial #: 0043-20-ASF, Israel Ministry of Health Authorization #202016632), and subsequently from all other participating centers. All participants provided written informed consent. The protocol was registered at ClinicalTrials.gov (ID #: NCT04595214).

Disclosures

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Conflict of Interest: All authors declare no conflict of interest.

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