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Development of customized fetal growth charts in twins

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6 **DEVELOPMENT OF CUSTOMIZED FETAL GROWTH CHARTS IN TWINS**
7

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36

40 **Condensation**

41 The growth of uncomplicated twin fetuses is influenced by parental variables and fetal gender and it is
42 reduced in comparison with singletons starting from 26-28 weeks onwards. This reduction is more evident
43 in monochorionic twins.

44

45 **Short version of the Title**

46 Fetal growth in twin pregnancies

47

48 **Abstract**

49

50 **Background.** Twin gestations are at significantly higher risk of fetal growth restriction in comparison
51 with singletons. Using fetal biometric charts customized for obstetrical and parental characteristics may
52 facilitate accurate assessment of fetal growth.

53 **Objective(s):** To construct reference charts for gestation of fetal biometric parameters stratified by
54 chorionicity and customized for obstetrical and parental characteristics.

55 **Study Design:** Fetal biometric measurements obtained from serial ultrasound examinations in
56 uncomplicated twin pregnancies delivering after 36 weeks of gestation were collected by 19 Italian fetal
57 medicine units under the auspices of the Società Italiana di Ecografia Ostetrica e Ginecologica. The
58 measurements acquired in each fetus at each examination included biparietal diameter (BPD), head
59 circumference (HC), abdominal circumference (AC) and femur length (FL). Multilevel linear regression
60 models were used to adjust for the serial ultrasonographic measurements obtained and the clustering of
61 each fetus in twin pregnancy. The impact of maternal and paternal characteristics (height, weight,
62 ethnicity), parity, fetal sex and mode of conception were also considered. Models for each parameter were
63 stratified by fetal chorionicity and compared to our previously constructed growth curves for singletons

64 **Results:** The dataset included 1781 twin pregnancies (dichorionic 1289; monochorionic diamniotic 492)
65 with 8923 ultrasonographic examination with a median of 5 (range 2-8) observations per pregnancy in
66 dichorionic and 6 in (range 2-11) monochorionic pregnancies. Growth curves of twin pregnancies differed
67 from those of singletons, and differences were more marked in monochorionic twins and during the third
68 trimester. A significant influence of parental characteristics was found.

69 **Conclusion(s):** Curves of fetal biometric measurements in twins are influenced by parental
70 characteristics. There is a reduction in growth rate during the third trimester. The reference limits for
71 gestation constructed in this study may provide an useful tool for a more accurate assessment of fetal
72 growth in twin pregnancies.

73

74 **Introduction**

75 Twin gestations are at significantly higher risk of fetal growth restriction in comparison with singletons,
76 and this may contribute to their increased incidence of the adverse perinatal outcome. Fetal smallness for
77 gestational age may affect one of both fetuses, with an overall incidence estimated at 5%-10% in
78 dichorionic and 15%-25% in monochorionic pairs ^{1, 2}.

79 On this basis an accurate sonographic assessment of fetal biometry is warranted with the aim of detecting
80 cases with substantial growth restriction or discordance, and accordingly guiding the antenatal care. In
81 clinical practice, singleton pregnancy reference charts for ultrasound biometry are often applied to
82 multiple gestations, since specific nomograms for intrauterine growth of twins are few and of uncertain
83 clinical validity. In humans this sounds biologically inappropriate as the growth potential of twins might
84 per se be reduced compared to singletons, being limited by the inability of a woman to cope in late
85 pregnancy with two fetuses growing each at the same rate of a singleton.

86 Most studies have in fact documented a progressive flattening of the fetal growth rate in comparison with
87 singletons starting from 28 to 32 weeks ³⁻⁸. However, some of these studies failed to differentiate between
88 dichorionic and monochorionic pairs or between uneventful and complicated pregnancies. Very recently
89 some Authors ⁸ have provided ultrasound biometry charts in a large group of normal twin gestations
90 showing a reduced growth rate in monochorionic compared to dichorionic sets. Notably in this study
91 parental factors have not been considered in constructing the nomograms. The use of nomograms
92 customized on the basis of parental factors and fetal sex has been proposed to assess intrauterine fetal
93 growth in singleton gestations. This method compared with standard reference charts has been proven by
94 some to be more efficient in identifying the true small fetuses who are at higher risk of perinatal
95 complications ⁹⁻¹¹.

96 The aim of this study was to produce the first longitudinal charts for fetal ultrasound biometry in
97 uncomplicated twins gestations customized for chorionicity and for parental factors.

98

100 **Methods**

101

102 **Study Population**

103 This was a retrospective multicentric study performed in 19 Italian units under the auspices of the Società
104 Italiana di Ecografia Ostetrica e Ginecologica (SIEOG, www.sieog.it). All the units had proven expertise
105 in sonographic assessment of fetal growth and were opted in by the steering committee of the study. Data
106 were obtained from the combined ultrasound and delivery databases of each unit for pregnancies delivered
107 between January 2010 and December 2015.

108 Inclusion criteria were: uncomplicated twin pregnancy of known chorionicity; dating by crown-rump
109 length in the first trimester; known pregnancy outcome; delivery at or beyond 36 weeks of gestation of
110 two live fetuses; birthweight > 5th centile for the national Italian charts ¹²; information available on
111 maternal and paternal height and weight, parity and ethnic group. Gestational age was calculated by CRL
112 of the larger twin using the equation of Robinson and Fleming ¹³. The diagnosis of chorionicity was based
113 upon the sonographic findings obtained at the first trimester (two placental sites or lambda sign with a
114 single placental site for dichorionic; T sign with a single placental site for monochorionic). At that stage
115 accurate labelling of the twins ¹⁴ (twin 1 or A vs twin B or 2) was carried out in accordance with placental
116 site (in case of dichorionic pregnancies with two distinct placental masses), fetal position (up and down;
117 right or left) or cord insertion (monochorionic or dichorionic pregnancies with a single placental mass).
118 Fetal sex was also noted later in pregnancy to facilitate labelling. The maternal weight recorded during
119 the first trimester at the time of the first antenatal visit was considered.

120 Exclusion criteria were: conception by heterologous assisted reproductive technology; fetal structural or
121 chromosomal anomalies; uncertain chorionicity; monoamnionicity; spontaneous or iatrogenic reduction
122 from a multifetal gestation; maternal smoking; drug use; occurrence of twin to twin transfusion syndrome
123 (TTTS) or twin anemia-polycytemia sequence (TAPS); pre-existing maternal disease such as
124 hypertension, diabetes, renal and autoimmune disorders; the development of obstetric complications such

125 as pre-eclampsia and gestational diabetes. All the units used the same criteria to define the above
126 mentioned pregnancies complications, according to the guidelines of the Italian National Institute of
127 Health (ISS) for pregnancy care¹⁵.

128 A gestational age interval between 16 and 36 weeks was considered. Longitudinal measurements were
129 required, with a minimum of two sets of measurements for each twin pregnancy. As this was a
130 retrospective analysis of routinely collected anonymized clinical data, no ethical committee approval was
131 necessary according to national regulations.

132 We decided to rely on Italian national standard birthweight charts¹² in order to select which twin
133 pregnancies were to exclude due a birthweight below the 5th percentile. In our country we lack customized
134 birthweight charts for twins.

135

136

137 **Ultrasound measurements**

138 Fetal measurements were all made in accordance with SIEOG guidelines¹⁶. The biparietal diameter (BPD)
139 and the fetal head circumference (HC) were measured from a cross-sectional view of the fetal head at the
140 level of the thalami, with an angle of insonation of 90° to the midline echoes, a symmetrical appearance
141 of both hemispheres, a continuous midline echo (falx cerebri) broken in middle by the cavum septum
142 pellucidum and no cerebellum visualized. The BPD was measured at the level of the thalami from the
143 outer to the inner edge of the fetal skull. The HC measurements included the outer edge of the proximal
144 calvarial wall and the outer edge of the distal calvarial wall. The abdominal circumference (AC) was
145 measured on a transverse section of the fetal abdomen, showing the stomach bubble, symmetric lower
146 ribs, and the umbilical vein at the level of the portal sinus. The femur length (FL) was measured in its
147 longest axis perpendicular to the transducer direction, with calipers placed at the ends of the ossified
148 diaphysis without including the distal femoral epiphysis. Estimated fetal weight (EFW) was calculated
149 using the Hadlock III formula, that incorporates HC, AC, and FL¹⁷.

150

151 **Statistical analysis**

152 Comparison of the characteristics between dichorionic (DC) and monochorionic diamniotic (MCDA)

153 pregnancies was performed using chi square test for categorical variables and t-test or Mann Whitney U

154 test for continuous variables, according to their distribution. For modelling growth curve trajectories of

155 the fetal biometric parameters evaluated we used linear mixed models. The data set considered were

156 hierarchical in nature and a random effect structure that incorporates in the modelling the correlation for

157 both twin-pair and fetus within twin pair was used. The covariates considered in the model as fixed effects

158 were gestational age and other variables potentially influencing the ultrasound measurements as paternal

159 and maternal height (expressed in cm), paternal and maternal weight (expressed in kg), ethnic group

160 (categorized as European, East Asian, Central African and North African)¹⁸, parity (categorized as

161 nulliparous or parous) and gender (categorized as male or female). We performed a logarithmic

162 transformation of gestational age for fitting the models. Using polynomial transformation of different

163 degrees or other method of transformation did not improve the statistical significance. Separate growth

164 curves were built for DC and MCDA twins. These were analyzed in comparison with the growth charts

165 for uncomplicated singleton pregnancies customised for fetal sex, obstetrical and parental characteristics

166 recently developed by SIEOG¹⁹. Week specific difference in biometric measurements between twins and

167 singleton pregnancies were evaluated by the Wald test. Statistical analysis was performed using SPSS

168 version 20 (SPSS Inc. Chicago, IL, USA) and R software packages (version 3.1.2, <http://www.R-project.org>).

170

171 **Results**

172

173 Complete ultrasound fetal biometric data were obtained from 1781 twin pregnancies including 1289

174 dichorionic (DC) and 439 monochorionic diamniotic (MCDA) gestations who fulfilled the inclusion

175 criteria. Overall 8923 ultrasonographic examinations were available (6640 in DC and 2463 in MCDA).

176 The median number of observations per twin pregnancy was 5 in DC (range 2-8) and 6 in MCDA (range

177 2-11). The characteristics of the study population are shown in Table 1. When compared to DC twins,
178 MCDA pregnancies showed a lower incidence of nulliparity ($p<0.001$), of conception by in vitro
179 fertilization ($p<0.001$), an earlier gestational age at delivery ($p<0.001$) and a lower birthweight ($p<0.001$).
180 No significant differences were found for any other feature considered.

181 Tables 2 to 5 show the fitted regression coefficients and their statistical significance for the biometric
182 variables considered. As expected, gestational age had a significant positive association with all biometric
183 parameters. For BPD, maternal weight ($p=0.003$) and fetal sex ($p<0.0001$) were the other associated
184 covariates in DC twins, while in MCDA only the effect of fetal sex ($p<0.0001$) resulted significant. For
185 HC, maternal weight ($p=0.005$), maternal height ($p=0.004$), paternal height ($p=0.0015$) and fetal sex
186 ($p<0.0001$) had a significant association in DC twins, while maternal height ($p=0.032$), paternal height
187 ($p=0.05$) and fetal sex ($p<0.0001$) were associated in MCDA twins. Maternal weight ($p=0.005$), maternal
188 height ($p=0.0029$) and fetal sex ($p<0.0001$) resulted significantly related to AC measurements in DC
189 twins, while maternal height ($p=0.029$) and fetal sex ($p=0.0027$) showed the same association in MCDA
190 ones. When the FL was analyzed the significant covariates were maternal height ($p<0.0001$) and paternal
191 height ($p<0.0001$) in DC and paternal height ($p=0.001$) in MCDA pregnancies. Since there was a small
192 number of pregnancies in the three non-European groups the data do not allow any comment on the effect
193 of ethnicity on size or growth in twins. The effect size of all the considered covariates in the construction
194 of the mixed regression models are reported in supplemental materials (Supplemental Tables 1-4).

195 Figures 1 and 2 present the growth curves of the biometric parameters considered in DC and MCDA twins,
196 respectively, compared to singletons. Singleton reference limits were constructed using our national
197 growth charts customized for parent characteristics, obstetrical history and fetal sex¹⁹. Similarly in Figure
198 3 the EFW of DC and MCDA twins were compared to singletons. In order to allow a comparison the same
199 covariates were used for singletons and twins (i.e. European ethnicity for both parents, parity 0, maternal
200 weight 60 kg, maternal height 160 cm, paternal height 180 cm, male fetal sex) and tables (Supplemental
201 Tables 5-9) were generated to allow centile comparison. To allow an easy calculation of the growth curve
202 percentiles in twin pregnancies with different combination of covariates we have created an Excel based

203 file (Additional file 1).
204 The growth curves of DC twin pregnancies appeared to differ significantly from those of singletons,
205 with the reference percentiles of each biometric parameter showing lower values along the whole gesta-
206 tional interval considered. The differences with singleton growth charts were more evident with advanc-
207 ing gestation (Figure 1). When the Wald test was applied to evaluate week-specific differences in the
208 biometric variables between singleton and DC twins, BPD measurements appeared different from 31
209 weeks ($p=0.05$), HC from 29 weeks ($p=0.04$), AC from 27 weeks ($p=0.05$) and FL from 34 weeks
210 ($p=0.03$) of gestation.

211 Similarly the growth curves of MCDA twin pregnancies appeared to differ significantly from those of
212 singletons, the reference percentiles of each biometric parameter showing lower values along the whole
213 gestational interval considered. Again, the differences with singleton growth charts became more evi-
214 dent with advancing gestation and for some parameter such as AC appeared to increase progressively
215 during the third trimester (Figure 2). Significant differences were evidenced for BDP from 30 weeks
216 ($p=0.03$), HC from 28 weeks ($p=0.05$), AC from 26 weeks ($p=0.04$) and FL from 34 weeks ($p=0.05$) of
217 gestation.

218 Comparing DC with MCDA pregnancies, the measurements of each biometric index appeared slightly
219 smaller in the latter group, with differences being statistically significant only for AC after 33 weeks of
220 gestation ($p=0.03$)

221

222 **Comment**

223 **Principal findings**

224 In a large population of uncomplicated dichorionic and monochorionic twin pregnancies we documented
225 a different growth pattern in comparison with singleton fetuses, with a flattening of the biometric curve
226 starting at 26-28 weeks of gestation for all biometric parameters. Differences with singleton charts were
227 larger in monochorionic twins, progressively increasing during the third trimester for some parameters
228 such as AC. Moreover, as previously shown in singletons^{19,20}, a relationship between fetal biometric data

229 and parental characteristic and fetal gender was documented in both dichorionic and monochorionic twins.

230 **Clinical and research implications**

231 The use of twin-specific customized growth charts for ultrasound biometry may allow a more accurate
232 assessment of the intrauterine biometry of twins for clinical purposes. In particular this approach may help
233 the provider in distinguishing cases of true fetal smallness among a subgroup of pregnancies whose
234 intrauterine growth potential compared with singleton is per se reduced. On this basis a precise
235 sonographic diagnosis of fetal growth restriction among twin gestations is considered as a cornerstone to
236 optimize their clinical management and to reduce the risk of adverse outcomes. Surprisingly, in common
237 practice the reference charts for the intrauterine growth of twins are very often those in use for the
238 evaluation of singletons. A recent theory has recently suggested that constraints to maternal metabolism
239 increase in pregnancy may limit fetal growth; this may further explain why the intrauterine growth rate in
240 twins might be reduced in comparison with singletons²¹. On this basis the construction of specific twin
241 size charts has been claimed by some as a more reliable tool to assess the intrauterine fetal growth in
242 multiple gestation^{5, 22-24}. In principle, adjusting for multiple pregnancy, thereby shifting the normal range
243 of fetal growth downward, has the potential to mask truly growth restricted twins and increase perinatal
244 morbidity from failure to recognize growth restriction. However, having selected as a reference standard
245 a large group of uncomplicated twin gestations delivered close to term with a fetal birthweight of both
246 twins above the 5th percentile of population standards, this should reduce if not abolish the risk of
247 overlooking or masking a fetal growth restriction of one of both fetuses using these charts. This is simply
248 because the biometric data used to produce these charts come from super healthy twin gestations; although
249 the fetal measurements may appear smaller than those of a singleton a good placental function is in fact
250 required to a normal twin to fit in our curves. The choice to exclude those twin pregnancies whose
251 birthweight of one of both fetuses was below the 5th percentile of population standards was made with
252 the aim of maintaining a low threshold to define fetal smallness in twins. Using the 5th percentile at 36
253 weeks or beyond (rather than the 10th percentile as in singletons) as the lower limit to define smallness at
254 birth should account for the reduced intrauterine size of normally growing twins compared to singletons.

255 At the same time it should limit the risk of overlooking fetal growth restriction of twins and considering
256 as biologically normal for two fetuses what is a pathologically reduced growth pattern²⁵.

257 We are aware that monochorionic and dichorionic pregnancies have different rates of IUGR and also that
258 the threshold of physiological intertwin discordance of biometric data is varies according to the
259 chorionicity On this point we feel that the use of growth reference charts which are customized for
260 chorionicity may help the clinician also in the interpretation of the intertwin discordance as it should more
261 accurately reflect the specific intrauterine growth pattern of dichorionic and monochorionic twin
262 gestations. In other words using a reference charts which have been specifically designed for dichorionic
263 and monochorionic twin pregnancies the clinician will be able to assess more accurately the degree of
264 intertwin discordance and to determine, in a clinical context, if this difference should be considered
265 physiological or pathological. We decided to include the measurements obtained from both twins at each
266 visit rather than select the measurement of the largest twin. We are aware of the potential risk of
267 downgrading the reference interval for fetal growth, and that this may eventually decrease the sensitivity
268 in detecting antenatally pathological fetal smallness. However, the strict inclusion criteria of our study
269 population should reduce the risk of overlooking fetal growth restriction.

270

271 Previous Studies

272 Some older studies have shown smaller values for all biometric parameters obtained sonographically in
273 twin gestations compared to singletons. Ong et al.⁶ assessed 884 twins between 1986 and 1999, and used
274 a single random measurement of the dataset to construct the intrauterine nomograms. In their series the
275 AC values of twins were smaller in comparison with singleton gestations only after 32 weeks of gestation,
276 whereas BPD appeared reduced along the whole pregnancy. However, in the aforementioned study the
277 fetal growth charts were not adjusted for the chorionicity, as the differentiation between dichorionic and
278 monochorionic placentae has become accurate only more recently.

279 In 2012 Liao et al.⁴ assessed a smaller group of 125 uncomplicated diamniotic twin gestations in a
280 longitudinal prospective study, without differentiating for chorionicity. Using a multilevel regression

281 approach they constructed specific charts for all biometric parameters, whose values appeared smaller
282 compared with those obtained in singleton after 28 weeks.

283 Stirrup et al.⁸, using a large database of twin pregnancies, retrospectively built reference charts for all
284 fetal biometric parameters from 14 weeks to term adjusting for chorionicity. Similarly to our findings,
285 they found that ultrasound measurements of fetal growth showed a significant reduction in twin
286 pregnancies, particularly in the third trimester, compared with singletons. Also in their cohort, this
287 reduction was more marked in MCDA gestations. However, they also included complicated twin
288 gestations such as those with twin to twin transfusion or fetal growth restriction, which contributed to the
289 construction of the reference growth charts. This should be acknowledged as a methodological limitation
290 in building the twin specific nomograms for the intrauterine fetal growth.

291 Recently ultrasound based estimated fetal weight reference charts have been retrospectively built in 642
292 uncomplicated dichorionic and monochorionic twin pregnancies⁷. In this study the reference centiles of
293 fetal weight were significantly lower among monochorionic compared to dichorionic twins along the
294 whole pregnancy. Furthermore, similarly to previous studies, a significant flattening of the intrauterine
295 fetal weight curve in twins compared to singleton in the third trimester was reported, starting earlier in the
296 monochorionic than in the dichorionic group (28 vs 32 weeks). Finally, a recent study from the National
297 Institute of Child Health and Human Development has shown that compared with singleton fetuses,
298 dichorionic twin fetuses have a progressively asymmetrical slower growth, beginning around 32 weeks
299 of gestation³.

300 In this study, as previously proposed by others^{26, 27}, we opted to customize all the fetal biometric
301 parameters obtained at ultrasound and not only the estimated fetal weight. We believe indeed that this is
302 a more appropriate approach when developing fetal growth charts as some parameters may vary according
303 to the ethnicity or the constitutional characteristics of the parents, these differences not being specifically
304 reflected by the changes in estimated fetal weight⁷. Some of our findings related to the association
305 between fetal biometric parameters and parental characteristics are not easy to interpret: HC has many
306 more significant associations than the BPD. This is not biologically plausible, and might easily be

307 explained by the fact that the variance in BPD measurements is smaller than for HC and thus may not
308 have a sufficient power to reveal associations of HC. Moreover, the fact that maternal weight does not
309 seem to affect significantly the fetal growth charts of twins, as opposed to singletons^{19, 20}, may be
310 explained by the fact that the mean maternal weight in twins is larger than in singletons.
311 The clinical usefulness of customization has been the object of debate in the last years^{10, 28, 29}. However
312 a number of publications have shown that in singleton pregnancies the use of customized growth charts
313 is more accurate in identifying the true small fetuses whose risk of perinatal complications is actually
314 increased.^{10, 11}. Also in multiple pregnancies, the use of customized birth weight charts for twins rather
315 than those for singletons seems more accurate in predicting adverse fetal and neonatal outcomes^{5, 24}.
316 Following the publication of the large prospective INTERGROWTH-21st study³⁰, which failed to
317 demonstrate a significant impact of the ethnicity on the variability of fetal biometric data, the use of
318 normative universal growth charts has been claimed as more appropriate. However the concept of an
319 optimal fetal growth pattern that should ideally be followed by each fetus has been challenged from a
320 theoretical point of view³¹. Moreover, recent evidence from singleton pregnancies suggests that the
321 INTERGROWTH-21st standards may be less effective than population³² or customized⁹ charts in
322 identifying those small fetuses at risk of perinatal mortality or morbidity. Similar evidence is currently
323 not available for twin pregnancies and also the current study does not allow to conclude that customized
324 biometric models, in comparison to population-derived reference ranges, perform better in terms of their
325 ability to identify individual fetuses at risk of adverse perinatal outcomes. The clinical usefulness of these
326 models should be evaluated in a clinical trial and only if they are shown to be superior should they be
327 considered for use in a clinical context. Altough such a validation can be carried also in retrospect, only a
328 prospective study would be able to provide a convincing demonstration that the use of customized charts
329 produces a measurable benefit in terms of reduction of perinatal morbidity or mortality compared to the
330 use of the standard curves, as recently shown for singletons⁹.

331 **Strengths and limitations**

332 The main strength of our study is that complicated twin pregnancies were excluded in order to construct
333 unbiased reference charts. In particular as the objective of this study was to build the normal intrauterine
334 biometric charts of healthy uncomplicated dichorionic and monochorionic twin gestations, we decided a
335 priori to exclude from the retrospective data collection the twins whose birthweight was below the 5th
336 centile for population standards, and those who were delivered before 36 weeks. This may affect the con-
337 struction of the reference interval and determine a selection bias between monochorionic and dichorionic
338 pairs. However the rationale for this choice was to avoid the data contamination with measurements ob-
339 tained from complicated twin pregnancies.

340 Thanks to a multilevel regression model that takes into account fixed and random effects, we adjusted our
341 curves for chorionicity, parental variables and fetal gender. In particular, a main difference from previous
342 studies is that these two latter factors, both constitutional variables of both parents and fetal gender, were
343 considered in the model and were shown to have a significant impact on the different fetal biometric data,
344 as previously documented in singleton²⁰. The decision to include also paternal variables seems biologi-
345 cally plausible due to the presumably relevant contribution of the father to the fetal growth potential;
346 however in this study the genetic paternity was based upon maternal report and remained unproven. Dif-
347 ferently from our previous study on singleton gestations¹⁹, no association was found between the twins
348 biometry and the parental ethnicity, although the lack of significance may depend on the low number of
349 non-European women enrolled in the current study.

350 The participation to this multicentric trial was arbitrarily restricted to units with long standing experience
351 in obstetric ultrasound whose operators are certified by the Italian Society of Ultrasound in Obstetrics and
352 Gynecology, and this is certainly a further strength point of this study. Finally this is to date the study with
353 the largest number of biometric data longitudinally collected in twins used to construct the nomograms of
354 intrauterine fetal growth.

355 Among the main weaknesses of this study it is the retrospective design which prevented us from validating
356 our growth curves in the clinical practice and assessing if this tool allows a more accurate assessment of
357 intrauterine twins biometry, thus reducing the risk of adverse perinatal outcome. However the design of

358 the study which by definition has retrospectively selected the largest group of normal uneventful twin
359 gestation to construct the reference charts did not allow to test the clinical usefulness of these customized
360 curves in the management of twin gestations. Moreover the rather homogenous racial mix, with
361 approximately 90% of women of European origin probably led to an underestimation of the effect of
362 parental racial origin on twin growth, which was not statistically significant for any biometric parameter.
363 Some population studies on the customization of twin birth weight charts have actually proven an effect
364 of maternal ethnicity on birthweight²³. The unavailability of the father or the lack of certainty on paternal
365 data is an additional limitation of our model which supports customization of fetal biometry in accordance
366 to the characteristics of both parents. We customized our growth curves according to the maternal weight
367 prepregnancy weight at the time of the first ultrasound scan. Altough the pre-pregnancy weight is a more
368 reliable index of maternal characteristics independently from the effect of the pregnancy, its exact value
369 may be uncertain or unknown to the woman; therefore we pragmatically decided to use the weight which
370 was actually measured by the midiwife and reported in the antenatal notes. Furthermore, availability of
371 the full set of study variables was a criterion of inclusion, and all participating centers shared only datasets
372 containing this information: we were therefore unable to analyze details on the exclusions and the number
373 of each type of exclusion in order to assess the representativity of the population.

374 The two fetuses within the twin pair were in fact treated as two independent fetuses and provided two
375 distinct set of measurement for each visit. However as previously suggested by others³³ the measurements
376 within a twin pair are not completely independent from each other and they are correlated to each other.
377 This is biologically consistent with the fact that we may sonographically diagnose chorionicity but not
378 zygosity and this latter factor may significantly affect the interdependency of the biometric data within a
379 dichorionic twin pair. On this base, the use of a regression mixed model which accounts for the correlation
380 of twin measurements has been suggested by some when assessing fetal biometry and growth of
381 dichorionic twin gestation. This has been done also in our study as specified in the methods section
382 although we acknowledge that this method, despite being widely used, cannot completely adjust for those
383 biological and environmental factors which determine the interdependency of the biometric data within a

384 twin pair.

385 We decided to include the dataset obtained from both twins at each visit rather than select the measurement
386 of the best twin. We are aware of the potential risk of downgrading the reference interval for fetal growth
387 and that this may eventually decrease our sensitivity in detecting antenatally a pathological fetal smallness.
388 However having built our curves with the biometric data of superhealthy uncomplicated dichorionic and
389 monochorionic twin gestations should keep high enough our reference interval reducing the risk of
390 overlooking fetal growth restriction ²⁵. Moreover, a recent analysis suggests that increasing intertwin
391 birthweight discordance is not associated with long-term neuropsychological disadvantages. However it
392 carries an increased risk of neonatal complications and infant mortality which might be, at least in part,
393 iatrogenic ³⁴. To this effect, our standards may help to better identify those discordant twins who may
394 benefit from increased intervention better than currently used standards.

395 A major issue remains whether fetal growth in twins should be measured against a singleton reference:
396 given the higher morbidity associated with twins, correcting for the presence of twins might not be
397 appropriate. However, there is evidence that optimal birthweights are different in twins and in singletons
398 ^{5, 23, 24}, and this justifies adopting specific size and growth references for twins. A prospective validation
399 study is needed to prove that our curves are superior to singleton or non-customized twin curves in clinical
400 practice.

401

402 **Conclusion**

403 In conclusion, this large retrospective study has confirmed that the intrauterine growth of uncomplicated
404 twin pregnancies is reduced in comparison with singletons starting from 26-28 weeks. This reduction is
405 more evident in monochorionic twins. The growth pattern of the fetal biometric parameters is significantly
406 influenced by parental variables and fetal gender. The reference ranges for gestation constructed in this
407 study may provide an useful tool for a more accurate assessment of fetal growth in twin pregnancies.

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485

486 Table 1. Characteristics of the twin pregnancies according to chorionicity. Data are expressed as
 487 mean±SD or No (%)

	Dichorionic twin N=1289	Monochorionic diamniotic twin N=492	P value
Mother			
maternal age (years)	34.23±5.48	32.63±5.25	0.679
nulliparous	963 (74.68%)	313 (63.16%)	0.001
height (cm)	165.53±6.15	165.01±6.01	0.236
weight (kg)	62.72±10.73	61.07±10.99	0.258
Ethnic group			
European	1192 (92.40%)	442 (89.7%)	
East Asian	9 (0.70%)	23 (4.8%)	
Central African	41 (3.20%)	9 (1.8)	
North African	47 (3.7%)	18 (3.7)	0.222
Conception by in vitro fertilization (IVF)	422 (32.37%)	54 (10.98%)	0.0001
Father			
height	177.48±8.28	177.08±6.70	0.355
Ethnic group			
European	1204 (93.4%)	443 (90.2)	
East asia	8 (0.6%)	24 (4.8%)	
Central African	27 (2.1%)	6 (1.1)	
North African	50 (3.9%)	19 (3.9)	0.16
Fetus/newborn			
gestational age at delivery (weeks)	37.36±0.710	36.7±0.65	0.001
Birthweight(g)	2648.37±308.34	2516.40±328.41	0.001
sex			
male	637 (49.4%)	227 (46.2)	
female	652 (50.6)	265 (53.8%)	0.216

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491 Table 2. Mixed regression models for biparietal diameter (BPD) in dichorionic and monochorionic
492 diamniotic twins.

493

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-158.23	0.76	209.25	0.0001
log gestational age	68.57	0.19	366.23	0.0001
mother weight	0.02	0.01	2.99	0.003
sex (female)	-0.71	0.13	5.39	0.0001
Monochorionic diamniotic				
Intercept	-157.64	0.73	217.42	0.0001
log gestational age	68.68	0.22	313.98	0.0001
sex (female)	-0.78	0.20	3.94	0.0001

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499 Table 3. Mixed regression models for head circumference (HC) in dichorionic and monochorionic
500 diamniotic twins.
501

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-591.74	7.72	673.18	0.0001
log gestational age	244.33	0.48	510.55	0.0001
mother weight	0.07	0.03	2.80	0.005
mother height	0.08	0.04	2.00	0.04
father height	0.07	0.03	2.44	0.015
sex (female)	-2.69	0.34	7.86	0.001
Monochorionic diamniotic				
Intercept	-622.31	12.41	50.42	0.0001
log gestational age	245.53	0.62	403.76	0.0001
mother height	0.14	0.07	2.16	0.032
father height	0.17	0.06	2.84	0.005
sex (female)	-3.72	0.76	4.93	0.0001

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505 Table 4. Mixed regression models for abdominal circumference (AC) in dichorionic and monochorionic
 506 diamniotic twins.
 507

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-646.97	8.51	76.06	0.0001
log gestational age	257.15	0.62	413.39	0.0001
mother weight	0.06	0.03	2.99	0.05
mother height	0.16	0.05	1.91	0.0029
sex (female)	-1.68	0.43	3.88	0.0001
Monochorionic diamniotic				
Intercept	-643.36	12.85	50.07	0.0001
log gestational age	255.82	0.79	324.81	0.0001
mother height	0.17	0.08	2.20	0.029
sex (female)	-2.10	0.94	2.22	0.027

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511 Tab 5 Mixed regression models for femur length (FL) in dichorionic and monochorionic diamniotic
512 twins.

Parameter	Estimate	Std. Error	t	p
Dichorionic				
intercept	-163,20	2,12	76,99	0,0001
log gestational age	60,17	0,13	469,35	0,0001
mother height	0,05	0,01	4,34	0,0001
father height	0,04	0,01	4,51	0,0001
Monochorionic diamniotic				
Intercept	-157,43	2,64	-59,585	0,0001
log gestational age	60,37	0,17	365,67	0,0001
father height	0,05	0,01	3,214	0,001

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516 **LEGENDS**

517

518 Figure 1: Estimated 5th, 50th and 95th percentiles for BPD (a), HC (b), AC (c) and FL (d) in DC twins
519 (red lines) as obtained from linear mixed models. Data are compared with corresponding reference
520 percentiles in singleton pregnancies (black lines). In both groups values were customized for the same
521 paternal and obstetrical covariates and for fetal sex.

522

523 Figure 2: Estimated 5th, 50th and 95th percentiles for BPD (a), HC (b), AC (c) and FL (d) MCDA twins
524 (blue lines) as obtained from linear mixed models. Data are compared with corresponding reference
525 percentiles for in singleton pregnancies (black lines). In both groups values were customized for the same
526 paternal and obstetrical covariates and for fetal sex.

527

528 Figure 3: Estimated 5th, 50th and 95th percentiles for estimated fetal weight in DC twins (**panel a** red
529 lines) and MCDA twins (**panel b** blue lines). Data are compared with corresponding reference percentiles
530 for in singleton pregnancies (black lines). In both groups values were customized for the same paternal
531 and obstetrical covariates and for fetal sex.