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Human embryonic curvature studied with 3D ultrasound in ongoing pregnancies and miscarriages.

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Abstract

Embryonic growth is often impaired in miscarriages. We hypothesise that derangements in embryonic growth result in abnormalities of the embryonic curvature. This study aims to create first trimester reference charts of the human embryonic curvature and investigate differences between ongoing pregnancies and miscarriages.

Weekly ultrasonographic scans from ongoing pregnancies and miscarriages were used from the Rotterdam periconceptional cohort and a cohort of recurrent miscarriages. In 202 ongoing pregnancies and 33 miscarriages first trimester crown rump length and total arch length were measured to assess the embryonic curvature.

The results show that the total arch length increases and shows more variation with advanced gestation. The crown rump length / total arch length ratio shows a strong increase from 8^{+0} to 10^{+0} weeks and flattening thereafter. No significant difference was observed between the curvature of embryos of ongoing pregnancies and miscarriages. The majority of miscarried embryos could not be measured. Therefore, this technique is too limited to recommend the measurement of the embryonic curvature in clinical practice.

Introduction

Ultrasonographic parameters, such as the crown rump length (CRL), embryonic volume (EV), and description of the Carnegie Stages are available for the clinical and scientific evaluation of embryonic growth and development.[1-3] Recently, we demonstrated that human embryonic growth trajectories are associated with estimated foetal weight and birth weight.[1] Several periconceptional maternal conditions, such as the folate status, age, smoking and alcohol use contribute to embryonic growth and development, birth weight as well as to adverse pregnancy outcomes, e.g., neural tube defects and miscarriages.[4, 5]

Impaired embryonic growth is often observed in pregnancies ending in a miscarriage, whereby the frequency of neural tube defects is approximately 10-fold increased.[6, 7] From this background we hypothesise that impaired embryonic growth and development is associated with an abnormal embryonic curvature.

Our first aim was to study first trimester the human embryonic curvature and to create reference charts using a multivariate Bayesian model as has been developed by our group.[8] A second aim was to study differences in the embryonic curvatures between ongoing pregnancies and pregnancies ending in a miscarriage.

Materials and methods

This observational study is embedded in the Rotterdam periconceptional cohort (Predict study), an ongoing prospective cohort study conducted at the Erasmus MC, University Medical Centre, in Rotterdam, the Netherlands.[9] Pregnant women who participated in this study in 2009 and 2010 were enrolled via the outpatient clinic of the department of Obstetrics and Gynaecology at the Erasmus MC and local midwifery practices. Since 2003 as part of our routine clinical care women with a history of recurrent spontaneous miscarriage received biweekly ultrasound scans. We included a cohort of these women visiting our outpatient clinic between 2008 and 2015 for analysis.

The methods used were the same for both cohorts. All participants signed a written informed consent and the local medical ethics committee approved the study protocol (METC 232.394/2003/177 – 9 November 2005, METC 3232.395/2003/178 – 9 November 2005, MEC 2004–227 - 15 October 2004).

All women with ongoing pregnancies and those ending in a miscarriage received weekly 3D scans between 6^{+0} and 12^{+6} weeks gestational age (GA). Only women less than eight weeks pregnant entered the study and twins were excluded.

Ultrasonographic data

Transvaginal ultrasonographic volumes were obtained with Voluson E8 ultrasound equipment (GE Medical Systems, Zipf, Austria) using a transvaginal probe (GE-probe RIC-6-12-D; 4.5–11.9 MHz). With regard to the safety aspects of first trimester ultrasound the thermal index (TI) and mechanical index (MI) were kept below 1.0, the examiners were qualified and experienced, the duration of the examination did not exceed 30 minutes, the as low as reasonably practicable (ALARP) principle was respected and 3D images were stored for offline evaluation in order to reduce the exposure to ultrasound as much as possible.[10] The 3D volumes were evaluated offline by projecting these on the screen of the ultrasound machine and were displayed in the multiplanar mode for analysis. The images were rotated in order to obtain a precise midsagittal view of the embryo in the A-plane resulting in an axial view in the B- and a coronal view in the C-plane (**Figure 1**). The data were stored and all measurements were performed offline as recently described by our group.[8] In the midsagittal plane were measured both CRL, used in clinical practice as the greatest length of the embryo,[11] and the total arch length (TAL) defined as the dorsal contour traced from the cranial calliper of the CRL to the caudal calliper (**Figure 2**). The CRL/TAL ratio is supposed to represent the calculated estimate of the embryonic curvature. Two observers randomly performed the TAL measurements in triplicate. The mean of three measurements was used for further analysis.

In the ongoing pregnancies data on pregnancy course and outcome were obtained from medical records.

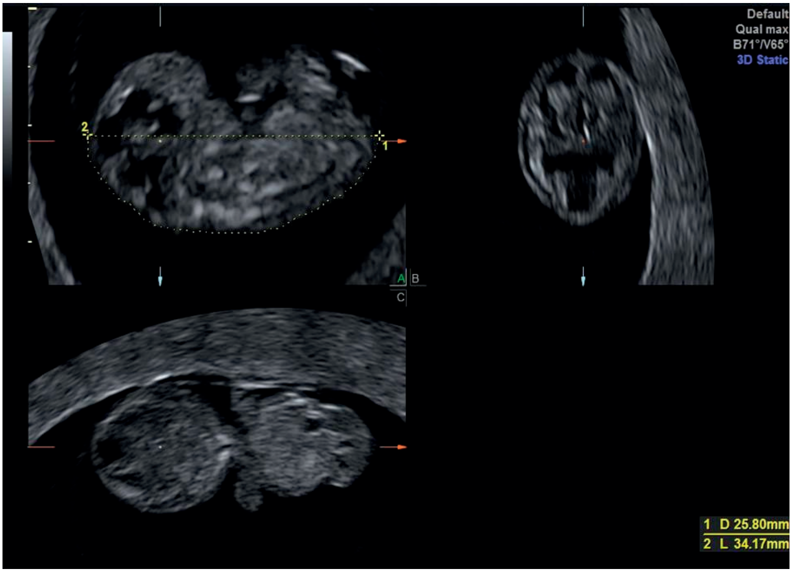

Figure 1: orthogonal triplanar image with crown rump length (CRL) and total arch length (TAL) measurements in the A-plane; A: sagittal view; B: axial view; C: coronal view

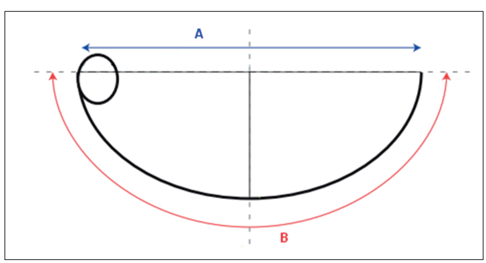

Figure 2: schematic representation of measurements; A: crown rump length (CRL), B: total arch length (TAL)

Pregnancy dating

According to our protocol, GA was calculated according to the first day of the last menstrual period when the woman had a regular cycle of 28±>3 days, we adjusted the GA for the duration of the menstrual cycle.[9] If the last menstrual period was missing or the difference of the GA determined by CRL and last menstrual period was more than 7 days, GA was based on the first CRL measurements after 8 weeks GA. In case of assisted reproductive technology, GA was determined by the date of oocyte retrieval plus 14 days in pregnancies conceived via in vitro fertilisation with or without intracytoplasmic sperm injection (IVF/ICSI), from the last menstrual period or insemination date plus 14 days in pregnancies conceived through intrauterine insemination, and from the day of embryo transfer plus 17 or 18 days in pregnancies originating from the transfer of cryopreserved embryos, depending on the number of days between oocyte retrieval and cryopreservation of the embryo.

Study population

From the Predict study we included 259 women enrolled in 2009 and 2010 (**Figure 3**) to create the reference charts of the embryonic curvature. We excluded one ectopic pregnancy, one pregnancy because of poor quality of the volumes, and 12 pregnancies with a discrepancy in GA of more than 6 days between CRL and the last menstrual period based on the Robinson curve or in case of an unknown last menstrual period. In the 245 pregnancies used for analysis we observed 43 miscarriages defined as a foetal death < 16 weeks GA. The 43 miscarriages from the Predict study were added to the 58 miscarriages derived from the recurrent miscarriage cohort, resulting in a total of 101 miscarriages. From this miscarriage cohort we had to exclude 9 pregnancies in which no images were obtained, 9 pregnancies with an empty sac and 50 pregnancies because of an early foetal demise resulting in a too small CRL for a precise TAL measurement. Of the resulting 33 miscarriages available for further analysis, 15 occurred after a history of recurrent miscarriages, defined as three or more miscarriages.

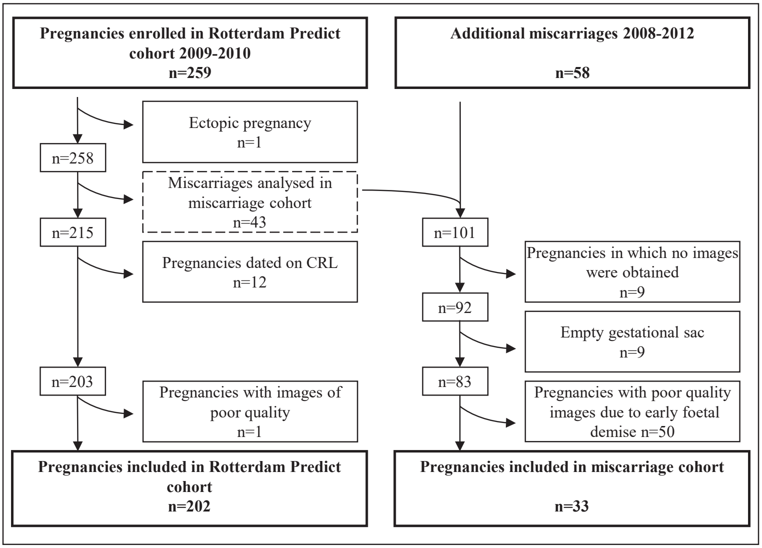

Figure 3: flowchart of study population

Statistical analysis

Maternal and pregnancy characteristics were summarised for the Predict cohort and the miscarriage cohort and compared between groups using the χ^2-test for categorical data, Student's *t*-test for normal distributions and the Mann-Whitney U-test for nonparametric continuous data.

Computations were performed with SPSS20 (IBM inc., Armonk, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). The standard deviation (SD) curves in **Figure 7** were computed with the R package gamlss,[12] assuming a model with a normal distribution, without transformation, and a spline for the mean curve and a linear relationship between the logarithm of the SD and GA.[8] The graph in **Figure 8** was made with the package ggplot2.

Reproducibility

The measurements of both CRL and TAL in the same volume were independently repeated three times and mean values were used for analysis. To assess intra- and interobserver reproducibility, a randomly selected subset of 30 first trimester volumes from 30 randomly selected pregnancies from the Predict cohort was measured a second time by the same examiner (SvG) and independently by another examiner (EvdM). For this purpose, five volumes were selected from each gestational week. Both examiners were blinded to the results of each other's measurements, each volume was unadjusted (raw data) and each measurement required manual adjustment of the volume to obtain the right image.

Results

General characteristics of ongoing pregnancies and miscarriages are shown in **Table 1**. In both groups the mean age (years ±SD) was comparable (32.1 ± 4.8 vs 33.7 ± 5.3 years respectively; *P* = 0.08). There was no significant difference between the groups in the number of women who were nulliparous (61.4% vs 48.5% respectively; *P* = 0.12) and conceived by means of in vitro fertilisation with or without intracytoplasmic sperm injection (30.7% vs 18.2% respectively; *P* = 0.14). In ongoing pregnancies after the first trimester, 5 (2.5%) pregnancies were terminated or ended in foetal or neonatal demise and 3 (1.5%) pregnancies resulted in a live born child with a congenital anomaly. Median GA at the moment of the first weekly visit was 7^{+0} (6^{+0} – 9^{+1}) in the ongoing pregnancy cohort and 6^{+5} (6^{+0} – 9^{+4}) weeks in the miscarriage cohort, with a median of 6 (range: 4-8) and 3 (range: 1-7) visits respectively per patient. We did not observe any neural tube defect in the study populations.

In 202 ongoing pregnancies and 33 miscarriages, a total of 1294 and 97 3D scans were performed respectively, of which in 1010 (78.1%) and 63 (64.9%) volumes, respectively, the image quality was sufficient to perform the measurements for the embryonic curvature. The median number of

	Predict cohort		Miscarriages		P
	(n=202)	Missing	(n=33)	Missing	
Maternal age, in years	32.1 ± 4.8	9	33.7 ± 5.3	0	0.08
Nulliparous	124 (61.4)	5	16 (48.5)	0	0.12
Conception via IVF/ICSI	62 (30.7)		6 (18.2)	0	0.14
Pregnancy outcome		0		0	
Miscarriage	-		33 (100.0)		
Termination of pregnancy	2 (1.0)		-		
Intra-uterine foetal death (>16wks)	2 (1.0)		-		
Neonatal death	1 (0.5)		-		
Livebirth	197 (97.5)		-		
Gestational age at delivery, in weeks^{+days} median (range)					
All pregnancies	39+3 (14^{+3} – 42^{+0})	1	-		
>24 weeks	39+3 (27^{+0} – 42^{+0})	0	-		
Birth weight (>24wks), in g	3305 ± 554	0	-		
Infant sex, male	95 (47.3)	1	-		
Congenital anomaly			-		
All pregnancies	6 (3.0)	1			
Livebirths	3 (1.5)	0			

Table 1: general characteristics of the Predict cohort and miscarriages included for the analysis, numbers are n(%) or mean ± standard deviation unless otherwise specified; IVF/ICSI, in vitro fertilisation with or without intracytoplasmic sperm injection

volumes in which we could measure the curvature was 5 (range: 2-7) in ongoing pregnancies and 1 (range: 1-5) in miscarriages. The reproducibility of the embryonic curvature is shown in **Table 2** and **Figure 4**. All intraclass correlation coefficient (ICC) values of inter- and intraobserver agreement were above 0.997, representing excellent reliability of the measurements between and within the two operators (**Table 2**). The Bland-Altman plots showed good agreement between the measurements as well (**Figure 4**).

In **Table 3** we illustrate that the percentages of ultrasound volumes in which the measurements for the curvature could be performed varied with GA. Between 8 and 11 weeks, more than 90% of volumes could be measured,

	Mean difference (%)	SD	ICC
Intra observer variability			
CRL	0.34	1.47	0.999
TAL	2.66	3.11	0.998
Inter observer variability			
CRL	-0.24	2.23	0.999
TAL	-0.05	4.31	0.997

Table 2: reproducibility of crown-rump length (CRL) and total arch length (TAL) measurements; ICC, intraclass correlation coefficient; SD, standard deviation

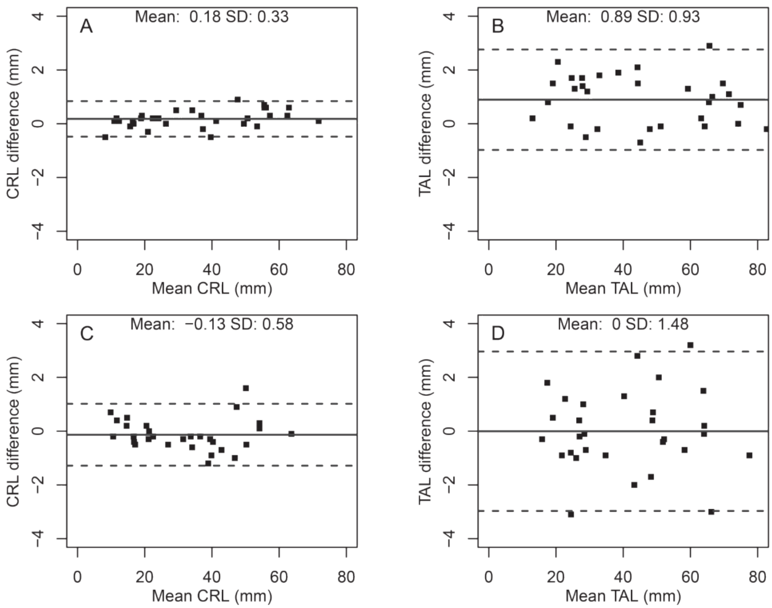

Figure 4: Bland-Altman plots of the intraobserver (upper figures) and interobserver (lower figures) variability for crown-rump length (CRL) and total arch length (TAL) measurements; SD = standard deviation

Week	All pregnancies (n=235)	%	Ongoing pregnancies (n=202)	%	Miscarriages (n=33)	%
6	34/141	24.1	32/127	25.2	2/14	14.3
7	132/207	63.8	117/178	65.7	15/29	51.7
8	195/214	91.1	175/190	92.1	20/24	83.3
9	188/202	93.1	174/186	93.5	14/16	87.5
10	179/191	93.7	174/185	94.1	5/6	83.3
11	165/191	86.4	161/187	86.1	4/4	100.0
12	138/182	75.8	136/179	76.0	2/3	66.7

Table 3: success percentages of total arch length measurements (measurements/ number of images; %) by gestational age (expressed in complete weeks)

whereas at 6 and 7 weeks this was only possible in 24% and 64% respectively. After 12 weeks, this percentage dropped to 76%.

Figure 5 shows the relation between TAL and GA. TAL increased and showed more variation with increasing GA. The relation between TAL and CRL is

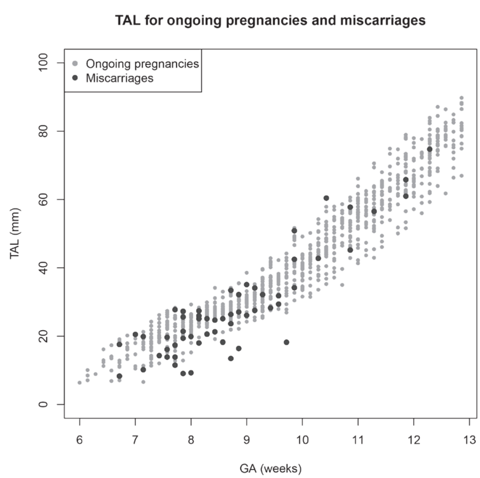

Figure 5: total arch length (TAL) versus gestational age (GA) for ongoing pregnancies and miscarriages

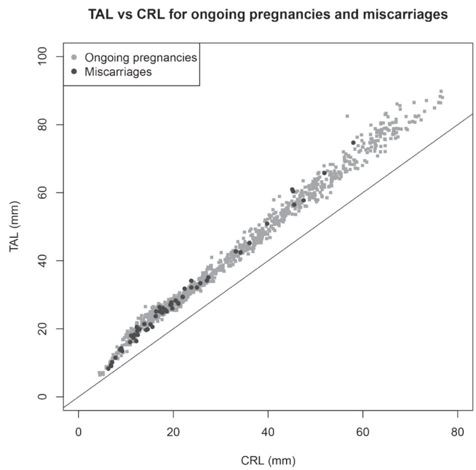

Figure 6: total arch length (TAL) versus crown rump length (CRL) for ongoing pregnancies and miscarriages

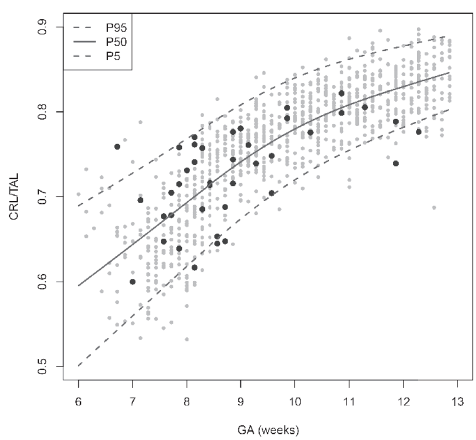

Figure 7: reference chart with percentile lines of the crown rump length crown rump length / total arch length ratio (CRL/TAL) versus the gestational age (GA); grey dots: ongoing pregnancies, black dots: miscarriages

shown in **Figure 6**. The CRL/TAL ratio represents the calculated estimate of the embryonic curvature. **Figure 7** displays the CRL/TAL ratio versus GA as reference chart, including percentile lines. The CRL/TAL ratio shows a strong increase at the beginning of the curve from 8^{+0} to 10^{+0} weeks, after which the curve flattens. This indicates that the embryo, being almost a circle in the very early stages, is gradually coming in a more linear, uncurved position mainly between 8 and 10 weeks GA. Longitudinal charts of the individual curvature of miscarriages are shown in **Figure 8**. No significant differences were observed in TAL and CRL/TAL between ongoing pregnancies and miscarriages.

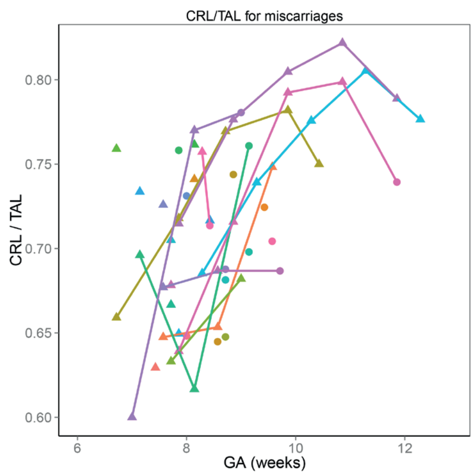

Figure 8: crown-rump length / total arch length ratio (CRL/TAL) versus gestational age (GA) plotted for individual miscarriages; triangles represent measurements in embryos with positive heart action and circles were used for measurements after embryonic demise

Discussion

In this prospective periconception cohort we have created reference charts of the human embryonic curvature in the first trimester of pregnancy in a study population derived from a tertiary care outpatient clinic. The data were analysed by means of a novel multivariate Bayesian model for longitudinal measurements.[8] This tailor made model provided a better fit than the existing univariate model.

We demonstrated that the first trimester curvature of the human embryo could be estimated reliably by measuring the CRL and TAL and calculating the CRL/TAL ratio. The embryonic curvature is positively associated with CRL and GA and decreases towards the end of the first trimester. Significant differences of the CRL/TAL ratio between miscarriages and ongoing pregnancies were not observed. Since we are the first to measure the curvature in both ongoing pregnancies as well as miscarriages, it will be difficult to estimate how many pregnancies ending in a miscarriage should be measured in order to observe a difference. Although we obtained volumes in 92 pregnancies ending in a miscarriage, the low feasibility in the miscarriage cohort precludes us from drawing firm conclusions. Therefore, the technique is too limited to recommend the measurement of the embryonic curvature and cannot be applied to the majority of women experiencing a miscarriage.

From studies on embryonic development it is known that the curved shape at about 7 weeks GA changes to an upright position at about 10 weeks.[11] This straightening mechanism, most likely caused by differences in growth rate of several embryonic structures, will influence the CRL measurement, as it is generally accepted to measure the greatest length in a straight line for this growth parameter. These changes are supported by our data, in which we observed a decrease in curvature in this period (**Figure 7**).

First trimester embryonic measurements are strongly determined by GA.[13] In order to reduce confounding by imprecise dating we therefore excluded pregnancies with a discrepancy in GA of more than 6 days between CRL and the last menstrual period. The repeated measurements were performed on ultrasound scans obtained between 6^{+0} to 12^{+6} weeks GA. The feasibility of the measurements appeared to be best between 8 to 11 weeks GA. The problems of imprecise measurements before 8 weeks and after 12 weeks appeared to be due to the small size of the embryos and the increasing artefacts due to foetal movements, respectively. No difference in embryonic curvature could be observed between ongoing pregnancies and pregnancies ending in a miscarriage, which might be due to the small number of miscarriages in which measurements could be performed.

Prenatal detection of congenital anomalies is gradually shifting to the late first trimester. The intracranial translucency and other signs and ratios in the first-trimester posterior brain have been used for the detection of spina bifida.[14] In this study, as a first step the feasibility of the estimation of the embryonic curvature is demonstrated. This might be of particular interest for the screening of specific anomalies of the spinal column, including neural tube defects, such as spina bifida at an even earlier stage. Prenatal detection of neural tube defects will depend on the type of the anomaly and the GA. We can only speculate about the question whether the CRL/TAL ratio in the future may contribute to an earlier detection of spina bifida, which in current clinical practice is diagnosed mostly in the second trimester of pregnancy.[15]

In mice, the *curly tail* (*ct*) mutation is associated with spina bifida. It is not clear whether the increased curvature associated with failure of closure of the posterior neuropore is the cause or consequence of the spina bifida aperta.[16] In other species, including man, a decreased curvature was related to an increase in closure of the posterior neuropore.[17]

We were not able to study possible changes in the curvature in association with neural tube defects, since we did not observe any neural tube defects sonographically and pathological examination of miscarriage tissue was not available. However, it would be interesting to further investigate the embryonic curvature with CRL and TAL measurements in spina bifida and other spinal column related anomalies in a large first trimester birth cohort with the pathological investigation of miscarriage tissues and stillbirths in the future.

This will also give opportunities to investigate associations between periconceptional maternal conditions, such as folic acid supplement use, and the development of the embryonic curvature.

In conclusion, the curvature of the first trimester human embryo can be determined reliably using 3D ultrasound measurements for CRL and TAL and by calculating the CRL/TAL ratio with an optimal time window of 8 to 12 weeks GA. The curvature of embryos resulting in a miscarriage was not significantly different from ongoing pregnancies. Our charts might be used in further studies on embryonic growth or on development of first trimester prenatal diagnosis of anomalies related to the spinal column.

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