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Assessment of human fetal cardiac autonomic nervous system development using color tissue Doppler imaging

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Abstract

Objectives: Functional development of the fetal cardiac autonomic nervous system (cANS) plays a key role in fetal maturation and can be assessed through fetal heart rate variability (fHRV)-analysis, with each HRV parameter representing different aspects of cANS activity. Current available techniques, however, are unable to assess the fHRV parameters accurately throughout the whole pregnancy. This study aims to test the feasibility of color tissue Doppler imaging (cTDI) as a new ultrasound technique for HRV analysis. Secondly, we explored time trends of fHRV parameters using this technique.

Methods: 18 healthy singleton fetuses were examined sequentially every 8 weeks from 10 weeks GA onwards. From each examination, 3 cTDI recordings of the four-chamber view of 10 seconds were retrieved to determine accurate beat-to-beat intervals. The fHRV parameters SDNN, RMSSD, SDNN/RMSSD, and pNN10, each representing different functional aspects of the cANS, were measured, and time trends during pregnancy were explored using spline functions within a linear mixed-effects model.

Results: In total, 77% (95% CI 66–87%) of examinations were feasible for fHRV analysis from the first trimester onwards, which is a great improvement compared to other techniques. The technique is able to determine different maturation rates of the fHRV parameters, showing that cANS function, presumably parasympathetic activity, establishes around 20 weeks GA and matures rapidly until 30 weeks GA.

Conclusions: This is the first study able to assess cANS function through fHRV analysis from the first trimester onwards. The use of cTDI to determine beat-to-beat intervals seems feasible in just 3 clips of 10 seconds, which holds promise for future clinical use in assessing fetal well-being.

KEYWORDS

cardiac autonomic nervous system, color tissue Doppler imaging, fetal development, heart rate variability, Ultrasound

1 | INTRODUCTION

Fetal heart rate variability (fHRV) is a fundamental marker for fetal well-being and therefore the cornerstone of fetal monitoring.[1,2] Fluctuations in fetal heart rate are regulated by the cardiac autonomic nervous system (cANS) in order to maintain cardiovascular homeostasis in the changing intrauterine environment.[3] In general, the cANS consists of a parasympathetic and sympathetic branch. Parasympathetic stimulation, mediated by the release and rapid hydrolysis of acetylcholine, decreases heart rate on short term, resulting in brief changes.[3,4] Sympathetic stimulation via (nor)epinephrine results in an opposite effect on a longer time frame.[3]

As alterations in heart rate reflect the modulation of sympathetic and parasympathetic activity, cardiac autonomic function can be assessed through analysis of heart rate variability (HRV).[1] HRV analysis is a noninvasive internationally standardized tool that quantifies the variation in intervals between consecutive heartbeats by measuring multiple HRV parameters, each representing different aspects of cANS activity.[1,5,6] In the mature human cANS under healthy and resting conditions, the sympathetic and parasympathetic branch interact in balance with a predominant vagal tone and alterations in HRV largely depend on vagal modulation.[4,7,8] In utero, fetal HRV parameters increase with gestational age reflecting the increasing maturation of the cANS.[9–12] The balance between parasympathetic and sympathetic activity differs throughout gestation as both branches show a different maturation rate.[13–15]

Generally, a considerable variation in fetal heart rate implies fetal well-being while decreased fHRV, a result of predominant sympathetic tone, is an early marker for fetal distress.[1,2] Uteroplacental dysfunction and chronic hypoxia are risk factors for perinatal morbidity and mortality and are associated with decreased fHRV.[16–20] A decreased fHRV has furthermore been suggested to precede intrauterine fetal demise.[21] Therefore, the assessment of the fetal cardiac autonomic function is an important indicator for the early identification of fetal compromise.[2] Furthermore, fetuses with congenital heart disease (CHD) show an altered function of the cANS whereas postnatally a disturbed function of the cANS is associated with arrhythmogenesis in surviving CHD patients.[22,23] Knowledge about the normal functional development of the cANS in utero is requisite to understand and recognize (dys)function of the cANS in pathologic conditions. Cardiac autonomic function during fetal life, however, remains difficult to examine in detail with the current available fetal surveillance techniques.

Previous studies attempted to identify the functional development of the cANS through fHRV analysis, yet the exact timeline of establishment of a functional cardiac innervation in utero is still not fully elucidated. Most fHRV data are derived from cardiotocography (CTG) registrations starting at the late second trimester, while literature on fetal beat-to-beat variability in early fetal stages is scarce. Moreover, CTG does not have the temporal accuracy to assess beat-to-beat variability.[24–26] Fetal magnetocardiography (fMCG) and electrocardiography (fECG) are also used in research setting.[27–32] Both techniques, however, are not suitable for daily clinical use as the former is too expensive and highly specialized and the latter encounters frequent signal loss.[24] A reliable accurate tool to assess fHRV to determine the function of the cANS could be beneficial in the clinical setting.

Color tissue Doppler imaging (cTDI) has the advantage of being an accurate, simple technique to extract beat-to-beat intervals with high intra- and inter-observer agreement.[33] FHRV analysis using cTDI has not been studied before. Therefore, this study aims to test the feasibility of using cTDI for fHRV analysis. In addition, we aimed to perform a first exploration of the longitudinal development of fHRV parameters using this technique.

2 | MATERIAL AND METHODS

This study was approved by the regional Medical Ethics Committee (NL65087.058.18) and conducted according to the principles of the Declaration of Helsinki (version 7, October 2013).

2.1 | Participants

Women with a spontaneous, singleton, and low-risk pregnancy were recruited between June 2018 and March 2019 for this longitudinal observational study. Women were recruited if they had a viable first-trimester ultrasound scan in a community based, primary care ultrasound center, or in our tertiary care hospital. Only subjects with signed written informed consent were eligible to participate. Fetal and maternal conditions that possibly influence fHRV were excluded, such as fetal congenital and/or chromosomal abnormalities, fetal rhythm disturbances, fetal growth <2.3 percentile, pre-eclampsia in current pregnancy, maternal substance abuse of nicotine, alcohol or drugs, maternal medication use with cardiac side effects as well as any serious underlying maternal medical condition or maternal age <18 years. Sequential subjects were assigned for a specific week between 10 and 17 weeks GA to have the first examination in order of inclusion, with GA based on first-trimester ultrasound. Hence, each week of gestation was represented by at least 2 subjects. Thereafter, the examination was repeated sequentially every 8 weeks until 38 weeks. As this was an explorative study, we included 21 subjects in total.

2.2 | Data acquisition

An experienced ultrasonographer performed all measurements using a Toshiba Aplio i-800 ultrasound machine with abdominal PVI475BX and PVT674 High Frequency convex transducers. The examinations started with measuring fetal growth according to the guideline of the Dutch Society for Gynaecology and Obstetrics (NVOG).[34] After 5 minutes in resting position (supine position or in slightly left lateral tilt to prevent aortacaval compression), beat-to-beat intervals were acquired using cTDI cine loops of the apical or basal cardiac

four-chamber view with insonation angle of <30°. Sector width, depth, gain, and zoom box were adjusted, so the heart covered approximately 60% of the image, to optimize the frame rates to at least 90 frames/s (Figure 1). This corresponds to a precision level of at least 0.01 seconds, which is sufficient for fetal HRV analysis (eg, if FHR is 140/min, one heart beat lasts 0.43 seconds). Although fHRV is ideally assessed in fetal active and passive state, we only recorded clips in absence of fetal movements and breathing as it affects the Doppler signal of cTDI. Each clip contained at least 10 seconds video material and was repeated 10 times. The examination time was limited to 30 minutes. Additional maternal and fetal characteristics concerning health, the course of the pregnancy, labor, and postnatal period were obtained by a questionnaire.

2.3 | Data analysis

Beat-to-beat intervals were obtained using the myocardial velocity curve generated by cTDI, with region of interest (ROI) placed around the total heart or around both atrioventricular (AV) -valves. An offline measurement software package (Canon Medical Systems) automatically labeled the positive (*S′*) and most negative peaks (*E′* or *A′*) on the myocardial velocity curve and thereafter calculated the beat-to-beat interval between the two positive peaks (*S′*-*S′*) as well as the two negative peaks (*E′*-*E′* or *A′*-*A′*) separately (Figure 1).

For each examination, we aimed to select 3 clips of exactly 10 seconds with the best recording quality of the myocardial velocity curve for fHRV analysis. Artifacts were removed, if present. Clips with artifacts were only accepted if the artifact comprised maximal two heartbeats. FHRV parameters were separately calculated for each of the 3 clips using the timing intervals between either the positive (*S′*-*S′*) or most negative peaks (*E′*-*E′* or *A′*-*A′*), depending on which peaks had the best registration quality. Mean fHRV values of the 3 clips were used for statistical analysis. The intra- and interobserver variability of measuring timing intervals with cTDI was previously tested by our research group with an excellent intraclass correlation of 0.98 and 0.94, respectively.[33]

2.4 | Fetal heart rate variability parameters

Time domain fHRV parameters that were calculated according to the HRV Task Force guidelines are outlined in Table 1.[1]

2.5 | Statistical analysis

Study population characteristics were described with common metrics: continuous variables with approximately normal distribution as mean (±SD), continuous variables with skewed distribution as median (range) and categorical variables as frequencies (percentage). Trends over time for fHRV parameters were estimated using linear mixed-effects models, including a random intercept and slope per subject and as covariates the week of examination, mean fetal heart rate (mFHR) and sex. This model takes the correlation between repeated measurements of the same subjects into account and is capable of handling different numbers and timings of examinations. All subjects with at least one assessable examination were included to avoid selection bias.[35] To model the time trend, we used natural cubic splines with knots at percentiles of the data and the number of knots chosen based on model fit (Akaike information criterion (AIC)).[36] The use of a spline regression model allows a variable to sudden change the slope at any arbitrary point. Furthermore, 95% confidence intervals (CI) were calculated for the fHRV parameters to quantify sampling uncertainty. All statistics were performed with R Statistical software version 3.6.3 (Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

3.1 | Study population

21 women who met the inclusion criteria were included in the study. We excluded, however, 3 participants (14.3%): one because of maternal diabetes gravidarum (4.8%), one because of fetal sacrococcygeal teratoma (4.8%), and one because of neonatal craniosynostosis

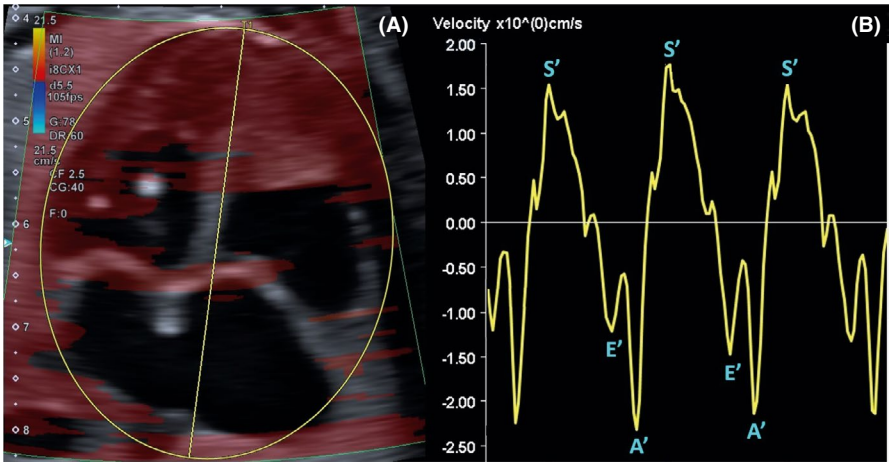

FIGURE 1 Schematic illustration of retrieving beat-to-beat intervals using color tissue Doppler imaging (cTDI). A, cTDI with placement of region of interest (ROI) around the total four-chamber view or around both atrioventricular (AV)-valves. B, Generated myocardial velocity curve showing longitudinal myocardial contraction (*S′*), passive ventricular filling (*E′*), and active atrial contraction in late diastole (*A′*)

TABLE 1 FHRV parameters

HRV parameter abbreviation	Formula	Meaning	Representing
SDNN	$\sqrt{\frac{\sum_{i=1}^{k}(NN_{i+1}-NN_i)^2}{k-1}}$	Standard deviation of all N-N beats (ms)	Total variability (in clips of 10 seconds mainly vagal activity)
RMSSD	$\sqrt{\frac{\sum_{i=2}^{k}(NN_{i+1}-NN_i)^2}{k-1}}$	Root mean square of successive differences between N-N beats (ms)	Parasympathetic control
SDNN/RMSSD ratio	$\frac{SDNN}{RMSSD}$	Ratio between SDNN and RMSSD	Sympatho-vagal balance
pNN10	$\frac{\sum_{i=1}^{N}\{\lvert NN_{i+1}-NN_i\rvert > 10ms\}}{N} \cdot 100$	Proportion of N-N intervals differing more than 10 ms [44]	Very short time variation regulated by parasympathetic activity

Note: N-N, normal-to-normal beats; *k* the total number of beat intervals in the individual dataset; *i*, index of summation; ms, milliseconds.

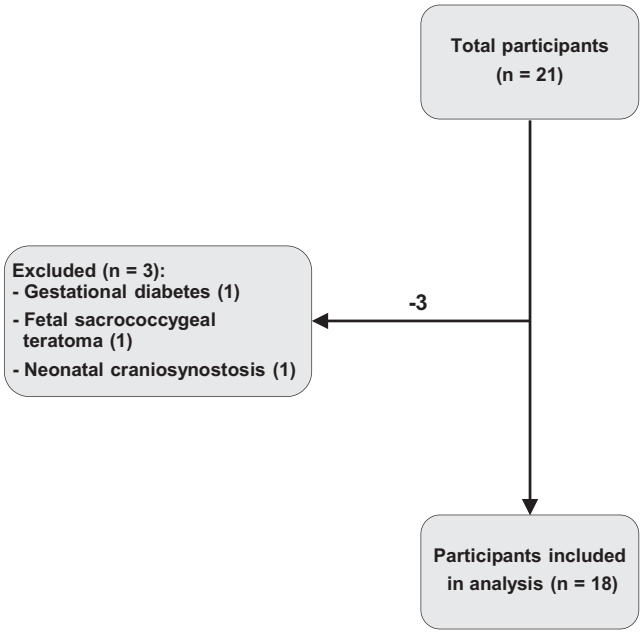

FIGURE 2 Flowchart subject inclusion

TABLE 2 Study population characteristics

Characteristics	Total n = 18
Maternal	
Age at birth	32.5 (± 3.9)
BMI	23.5 (± 2.9)
Gravidity	1.9 (± 1.0)
Parity	0.0 (0.0–2.0)
Fetus/neonate	
GA at delivery	40.5 (36.0–41.3)
Birth weight	3635 (± 598.9)
Sex	
Male	9 (50%)
Female	9 (50%)

(4.8%). This resulted in a total of 18 subjects eligible for analysis (Figure 2). Each week of gestation was represented by 2 subjects, except for the participants started at 13 and 15 weeks as they comprised 3 subjects. Maternal and fetal baseline characteristics are depicted in Table 2. Mean maternal BMI was 23.4 (SD 2.9). All fetuses and neonates had a weight >p2.3, and no peripartum complications occurred.

3.2 | Feasibility

A total of 64 examinations were obtained, of which 49 (77%, 95% CI 66–87%) had sufficient quality for HRV analysis. 44 of the examinations contained 3 suitable clips, while only 2 suitable clips could be retrieved in the remaining 5 examinations (8%). The examinations containing 2 suitable clips were considered as usable since these clips represented the fetal state during the examination in all cases. Reasons why HRV analysis was not possible in the 15 examinations with insufficient quality were as follows: too early pregnancy stages (<13 weeks) when quality of cTDI registration appeared very poor (5 examinations; 8%); a technical storage error (3 examinations; 5%) and poor registration of the S', E', and A' peaks in the myocardial velocity curve because of fetal or maternal movements (7 examinations; 11%). The registration quality was independent of BMI ($P = .2$). HRV analysis was performed mostly with S'-S' (45%) or A'-A' timing intervals (45%), while E'-E' intervals were only used in 10% of examinations. Mean recording time per examination was 22.6 minutes (SD 7.5), while mean recording time of only the selected clips was 8.7 minutes (SD 7.6). Mean frame rate per second (fps) was 115 (SD 27.1). Examinations at advanced gestational ages showed the lowest frame rates.

3.3 | Time trends of fHRV parameters

MFHR was 144 beats/min (SD 6.7) and showed a linear decreasing trend ($R^2 = 0.231$, $P = .0005$) over gestational age. Time trends for other fHRV parameters are depicted in Figure 3. Spline function with 2 or 3 knots was identified as the best model to depict SDNN, RMSSD, and SDNN/RMSSD ratio, whereas no knots (a linear trend) displayed pNN10 best. Both SDNN and RMSSD showed an increase from approximately 20–30 weeks GA. No particular trend was observed before and after these gestations. The SDNN/RMSSD ratio

increased until 26 weeks GA, yet this incline ceased thereafter. The SDNN/RMSSD ratio remained predominant to RMSSD (ie, <1), indicating a dominant vagal tone throughout the whole pregnancy. Only pNN10 showed a constant linear increment from 13 to 38 weeks GA.

4 | DISCUSSION

This is the first study that was able to assess accurate HRV trends in the fetus, as defined by the HRV Task Force guideline, from late first trimester onwards.[1] We used cTDI as a new technique to assess fHRV, measuring beat-to-beat intervals in its generated myocardial velocity curve, which was feasible in 77% (95% CI 66–87%) of all examinations (84% from 13 weeks onwards). SDNN, RMSSD, and SDNN/RMSSD ratio were shown to follow a nonlinear trend during fetal development, indicating that this new approach is able to determine different maturation rates of the cANS. SDNN and RMSSD increased from 20 weeks GA until 30 weeks GA, whereas no particular trend was observed before 20 weeks GA and after 30 weeks GA. This suggests that cANS activity, presumably mostly parasympathetic modulation, establishes around 20 weeks GA and that the parasympathetic branch matures rapidly until 30 weeks GA.

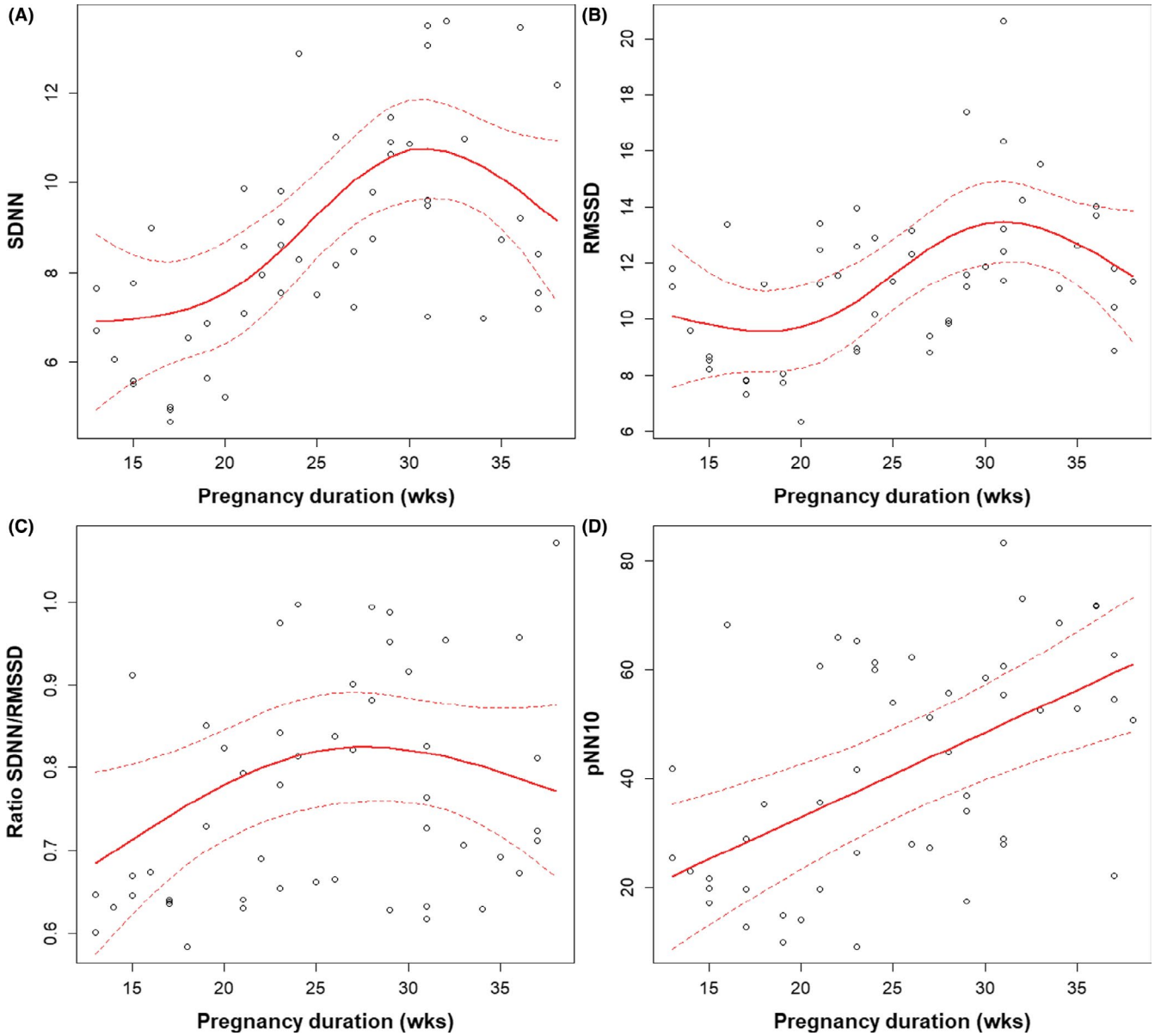

FIGURE 3 Time trends of fHRV parameters using spline function in linear mixed model (A-C) and linear regression (D). A/B, Both SDNN (representing total variability, mainly parasympathetic activity) and RMSSD (representing vagal control) increase from 20 to 30 weeks GA. This could imply that cANS activity, presumably mostly from the parasympathetic branch, establishes around 20 weeks GA and that the parasympathetic branch matures rapidly until 30 weeks GA. C, The ratio SDNN/RMSSD is always in favor of RMSSD, indicating a dominant vagal control throughout the pregnancy. D, pNN10 (marker for vagal modulation) increases linearly during the study period, suggesting vagal effect intensifies with advancing GA

4.1 | Feasibility

Our study shows that, with the use of cTDI, only 11% of examinations are not applicable because of poor signal-to-noise ratio and that fHRV trends can already be assessed from 13 weeks GA onwards. This is a great improvement compared to other techniques. FECG registration experiences frequent signal loss resulting in a low feasibility of 14%–63%, especially at early second trimester and between 28 and 34 weeks GA (0%–34%).[31,37] Although fMCG achieves a high feasibility at late second and third trimester (87%–100%), this technique encounters a poor signal-to-noise ratio in early stages.[38] Furthermore, the clinical relevance of fMCG is still debated as it remains too expensive and specialist-driven. Currently, CTG is the most used technique to assess fHRV. Although simple and inexpensive, CTG interpretation is limited by the lack of possibility to extract accurate beat-to-beat intervals as CTG converts beat-to-beat intervals every 0.25 seconds to an averaged fetal heart rate value.[24] This limits its use for computerized fHRV analysis.[25] In contrast, cTDI achieves a high accuracy with a mean of 115 fps in the current study, meaning that the mean precision by which fetal heart beats could be determined was 0.0087 seconds. With a mean fetal heart beat interval of 0.42 seconds, the established precision level of 2% is considered sufficient to demonstrate differences throughout the pregnancy (eg, SDNN established an increase of 50%). Although timing intervals generated by cTDI could possibly be affected by altered cardiac loading conditions as the myocardial velocity curve represents cardiac function, the current study is conducted in healthy, resting fetuses with recordings of only 10 seconds. Cardiac function is, therefore, expected to be consistent during one recording clip. Moreover, 90% of examinations is analyzed with S'-S' or A'-A' intervals. The S' peak represents longitudinal myocardial contraction, and the A' peak illustrates active atrial contraction in late diastole, which are considered to be stable in our recordings. The E' peak, representing passive ventricular filling, could be affected more by heart rate, loading conditions, or (early) cardiac dysfunction, yet these conditions are also expected to be consistent during the short recordings. CTDI measurements are easy to obtain as it only requires recordings of the cardiac four-chamber view, which is part of routine obstetric ultrasound examinations. The placement of the ROI is simple and fast with high intra- and inter-observer agreement, and the beat-to-beat intervals are automatically extracted.[33] The use of cTDI for fHRV analysis seems, therefore, a reliable substitute for daily clinical use.

4.2 | Development of fHRV parameters during pregnancy

We observed an increase of SDNN (total variability, in these short recordings mainly parasympathetic activity) and RMSSD (vagal control) from 20 weeks GA, while fHRV parameters remained continuously low prior to 20 weeks GA. Morphological studies show that terminal innervation is present in the human fetal heart from 18 weeks GA and that vagal sensory afferent nerves invade the brainstem from 20 weeks GA.[39,40] We therefore hypothesize that the function of the cANS, presumably mostly parasympathetic modulation, establishes at approximately 20 weeks GA. This is supported by studies using PW Doppler in the descending aorta, where fHRV also remained unchanged until 20 weeks GA.[41] In contrast, studies administering autonomic neurotransmitters describe a fetal cardiac reaction from 15 to 17 weeks GA onwards, yet this does not prove the endogenous function of the cANS as the fetal heart rate of preinnervated embryos also reacts to administration of autonomic neurotransmitters.[42,43] With the assessment of fHRV, we study the genuine in vivo function of the cANS, which seems to establish from 20 weeks GA.

The observed increment of SDNN and RMSSD between 20 and 30 weeks GA implies that the fetal cANS, presumably the parasympathetic branch, matures rapidly at this developmental stage. This is in accordance with recent findings.[15,37] It has been reported that parasympathetic control dominates the autonomic maturation between 20 and 30 weeks GA, resulting in the appearance of short-term heart rate variability, and the sympathetic branch dominates from 30 to 32 onwards illustrated by the appearance of long-term accelerations.[13] We could only confirm the predominance of parasympathetic control before 30 weeks, illustrated by the SDNN/RMSSD ratio in favor of RMSSD, reflecting vagal control. Sympathetic activity remains challenging to assess with this study as the recordings last only 10 seconds. The sympathetic branch is, furthermore, mainly activated by fetal movements, whereas cTDI recordings require absence of fetal movements. After 35 weeks, a second phase of parasympathetic development provoked by respiratory sinus arrhythmias (RSA) has been proposed.[15] With no particular trend of RMSSD, we were unable to identify this secondary developmental stage, presumably because fetal breathing movements, which are associated with RSA, were excluded during the recordings. Interestingly, pNN10 increased constantly from 13 to 38 weeks GA like previously reported, which can be explained by the linear decrease of mFHR.[15,37] As pNNx illustrates vagal modulation, vagal effect seems to intensify during prenatal development. However, RMSSD is a more accurate marker and therefore represents vagal activity best.

Although our data are comparable with recent findings, other studies showed variable results and therewith comparing data remains difficult.[15,37] Most differences in fHRV parameters are explained by the difference in fetal state. Another explanation might be different used methods. Most studies did not assess the development sequentially, while it is known that fHRV is more consistent intra-individually than inter-individually.[28] Moreover, studies with longitudinal measurements presented their data as linear regression or comparison between predetermined gestational age groups. The advantage of the current study is that time trends of the fHRV parameters were explored longitudinally, which is for the first time performed using spline functions within a linear mixed-effects model. The use of a spline regression model allows a variable to suddenly change the slope at any arbitrary point. As we observed that all time domain fHRV parameters, except for pNN10, do not follow a linear trend, the sudden changes in development might be missed or displaced when standard linear regression or comparison

between predetermined gestational age groups is used. Moreover, all our recordings were performed under the same circumstances. Lastly, some studies did not take a potential effect of sex or mFHR in account, whereas our study corrected for mFHR and sex.[12]

4.3 | Limitations

We are aware that our data need to be interpreted with caution as it is derived from only a small study group, primary aimed at determining the feasibility of cTDI for fHRV assessment. FHRV analysis by cTDI has the limitation that it can only be performed in fetal quiescence with short recordings, which means that relevant information about fetal autonomic development, in particular sympathetic development, could potentially be missed. This is congruent with postnatal HRV analysis, where the technique is more suited to identify parasympathetic than sympathetic tonus. Notably, dysfunction of the cANS is mainly the result of decreased parasympathetic activity and can, therefore, be distinguished with the current method.

4.4 | Implications

As cTDI seems a feasible technique for fHRV analysis, future studies with larger sample size are needed to examine whether this technique could distinguish between a normal or dysfunctional cANS, like in fetal distress or congenital heart disease. If so, cTDI recordings are easily acquired by any obstetrician in just 3 clips of 10 seconds, holding promising potential for future clinical use.

5 | CONCLUSIONS

In conclusion, use of cTDI to determine accurate beat-to-beat intervals for fHRV analysis is feasible from the first trimester onwards, yet only in fetal quiescence. The technique is able to determine different maturation rates of the fHRV parameters, showing that function of the cANS, presumably parasympathetic activity, seems to establish around 20 weeks GA and matures rapidly until 30 weeks GA. CTDI recordings are easy to obtain in only 3 clips of 10 seconds, which is promising for future clinical use. Whether this technique is capable to distinguish between a normal or dysfunctional cANS, like in fetal distress, remains to be determined.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Data available on reasonable request from the authors.

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