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Reference values for fetal heart rate monitoring in a large tertiary hospital population: a retrospective study

Chantal Eenkhoorn[a], Tom G. Goos[b,c], Arie Franx[a], Jenny Dankelman[c], Sten P. Willemsen[d] and Alex J. Eggink[a]

[a]Department of Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; [b]Department of Neonatal and Pediatrics Intensive Care, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; [c]Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands; [d]Department of Biostatistics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

ABSTRACT

Objective: To establish reference values for fetal heart rate (FHR) indices across time, frequency and nonlinear domains throughout pregnancy in a tertiary hospital population, considering sex. The influence of the number of fetuses, birth weight ,and time to delivery on FHR was evaluated.

Methods: This retrospective cohort study analyzed the initial FHR tracing upon hospital admission between 24° and 41° weeks of gestation, excluding cases in labor, with medication use, or a confirmed medical indication. Reference values were established using the Generalized Additive Models for Location Scale and Shape framework. Likelihood ratio test assessed whether including clinical variables significantly improved model fit.

Results: The cohort included 3219 fetuses, of which 48% were female and 91% singleton pregnancies. Median gestational age was 32^{+6}. Birth weight was below p10 in 22% and above p90 in 9%. Median tracing duration was 42.5 min and median signal loss was 1.95%. Most indices were significantly associated with gestational age and several showed significant sex differences. Model fit significantly improved for multiple indices when including number of fetuses, birth weight, or time to delivery.

Conclusions: This article presents gestational age- and sex-specific reference values for FHR in a large tertiary hospital population. The influence of gestational age was reaffirmed and significant differences related to sex, number of fetuses, birth weight, and time to delivery were identified. This enhances understanding of fetal autonomic regulation and supports a more individualized approach to predictive fetal monitoring. Further research is needed to determine the clinical utility of these reference values in practical monitoring and risk assessment.

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KEYWORDS
Computerized cardiotocography; fetal heart rate variability; GAMLSS; gestational age; reference values; sex differences

Introduction

Antepartum cardiotocography (CTG) is used to assess fetal wellbeing in high-risk pregnancies. In the Netherlands, CTG is performed in obstetrician-led care from 24 weeks of gestation. Classification guidelines have been developed specifically for intrapartum monitoring and do not offer guidance for use during the antepartum period. Conventional guidelines dictate a visual assessment of key features of the CTG, followed by a classification based on these features [1]. A Cochrane review found insufficient evidence that antepartum CTG improves perinatal outcome [2]. Visual assessment is subject to inter-observer and intra-observer variability and provides only information about the temporal characteristics of the fetal heart rate (FHR) [3].

The FHR is regulated by the autonomic nervous system, which matures throughout pregnancy, shifting its balance from sympathetic to parasympathetic dominance [4]. Heart rate variability reflects autonomic activity and may be an important marker of fetal wellbeing [5]. Computerized CTG allows

CONTACT Chantal Eenkhoorn c.eenkhoorn@erasmusmc.nl Department of Obstetrics and Gynecology, Erasmus MC, Wytemaweg 80, Rotterdam 3015CN, The Netherlands

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for a more objective and comprehensive analysis of FHR variability by examining time-domain (beat-to-beat intervals), frequency-domain (signal power), and nonlinear-domain (signal complexity) features. These features, derived from adult research [6], provide deeper insights into fetal autonomic function.

Studies investigating the ability of computerized CTG to discriminate between healthy and pathological fetal conditions have shown promising results [5]. Developments in the field of neonatology have demonstrated the value of predictive monitoring through heart rate variability, as demonstrated by the HeRO monitoring system (Medical Predictive Science Corporation, Charlottesville, VA) for early prediction of neonatal sepsis [7]. A similar predictive approach in fetal monitoring may enable early prediction of fetal distress by identifying subtle changes in heart rate variability.

This study aims to establish reference values for FHR indices throughout pregnancy in a tertiary hospital population, considering sex. The effect of birth weight, number of fetuses, and time to delivery on the FHR indices will also be studied.

Materials and methods

This retrospective cohort study analyzed the initial FHR tracing of each woman on admission to the Department of Obstetrics and Gynecology of the Erasmus MC Sophia Children's hospital (Rotterdam, The Netherlands). Women admitted between July 2017 and December 2021, with a gestational age between 24° and 41° weeks and who underwent antepartum FHR monitoring were eligible for inclusion. To ensure FHR monitoring was performed antepartum, women were excluded if their first FHR tracing was performed within two days of delivery. Additional exclusion criteria were medication use within 48 h prior to FHR monitoring and confirmed medical conditions, known to affect FHR (detailed in Figure 1). Data were collected from the electronic health record (HiX, Chipsoft, Amsterdam, The Netherlands).

FHR monitoring was performed with the Avalon FM30 (Royal Philips NV, Amsterdam, The Netherlands). To address artifacts and signal loss from transducer misplacement, fetal movement, or maternal–fetal interference, preprocessing was applied to enhance signal quality. First, the start of the measurement was determined as the first value that was within 20% of the median FHR of the first 10 min of valid values, which also had to be greater than 110 bpm to exclude maternal–fetal confusion. Second, FHR values below 50 bpm and above 220 bpm were considered as missing data. Third, potential artifacts were identified: missing data were temporarily substituted with the preceding entry and the signal was segmented based on consecutive heart rate values that differed by more

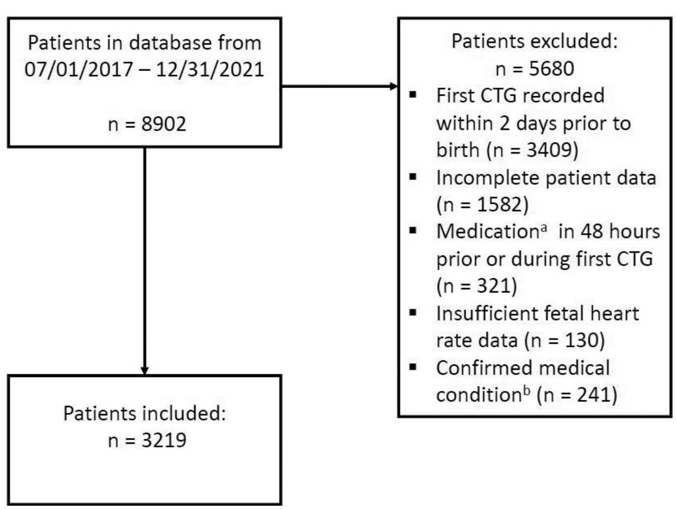

Figure 1. Flowchart of the study population. [a]Excluded medications: betamethasone, labetalol, lorazepam, magnesium sulfate, methyldopa, nifedipine, pethidine, temazepam, or atosiban. [b]Excluded medical conditions: preeclampsia, hypertension, chromosomal aberration, circulation problems, hematological disorder, infection, congenital anomalies, and preterm premature rupture of membranes. Abbreviation: CTG, cardiotocogram.

than 25 bpm. Segments with a duration of 60 s or less and segments with a median FHR that differed more than 25% from the overall median were considered as potential artifacts. If the median FHR of these segments differed by more than 20% from the median of the previous segment of the same length, the segment was identified as an artifact and replaced with missing values. This procedure was performed both forward and backwards. Last, cubic spline interpolation was used to fill data gaps of less than 20 s only if the FHR value before and after the gap differed by less than 25 bpm.

Next, time-domain, frequency-domain and nonlinear-domain heart rate indices were calculated. All heart rate indices and their definitions are presented in Table 1 and Appendix S1. In the time-domain analysis, the following indices were included: baseline, standard deviation (SD), root mean square of the standard deviation (RMSSD), SD/RMSSD, short-term variability (STV), interval index (II), long-term variability (LTV), long-term irregularity (LTI), average acceleration capacity (AAC), average deceleration capacity (ADC), acceleration phase-rectified slope (APRS), deceleration phase-rectified slope (DPRS), kurtosis, and skewness. To calculate the frequency-domain indices, a power spectral analysis was performed using a Hann window with sequences of length 512 and an overlap of 62.5%. The following frequency-domain indices were included: normalized power in the very low (VLFn; 0–0.03 Hz), low (LFn; 0.03–0.15 Hz), movement (MFn; 0.15–0.5 Hz), and high

Table 1. Heart rate indices.

Metric	Definition	Block length
Time domain		
AAC	Average acceleration capacity is an integral measure of all periodic acceleration-related oscillations	5-minute
ADC	Average deceleration capacity is an integral measure of all periodic deceleration-related oscillations	5-minute
APRS	Acceleration phase-rectified slope describes the average increase in fetal heart rate and time length of the increase.	5-minute
Baseline	Baseline fetal heart rate is the mean level of the fetal heart rate when accelerations and decelerations are excluded	1-minute
DPRS	Deceleration phase-rectified slope describes the average decrease in fetal heart rate and time length of the decrease	5-minute
II[a]	Interval index quantifies a coefficient of variation and is defined as the standard deviation of consecutive fetal heart rate samples divided by the short-term variability	1-minute
Kurtosis	Kurtosis measures the peakedness of the fetal heart rate distribution	5-minute
LTI[a]	Long-term irregularity quantifies the interquartile range of the distribution of the modal	5-minute
LTV[a]	Long-term variability quantifies the difference between the maximum and minimum fetal heart rate value	1-minute
RMSSD	Root mean square of the standard deviation of consecutive fetal heart rate samples	1-minute
SD	Standard deviation of consecutive fetal heart rate samples	1-minute
SD/RMSSD	Ratio of the standard deviation and root mean square of the consecutive fetal heart rate samples	1-minute
Skewness	Skewness measures the deviation of symmetry of the fetal heart rate distribution	5-minute
STV[a]	Short-term variability quantifies the absolute mean FHR difference on a sample-to-sample basis	1-minute
Frequency domain[b]		
VLFn	Normalized power in the very low frequency band [0 0.03]	5-minute
LFn	Normalized power in the low frequency band [0.03 0.15]	5-minute
MFn	Normalized power in the movement frequency band [0.15 0.5]	5-minute
HFn	Normalized power in the high frequency band [0.5 1]	5-minute
LF/(MF + HF) ratio	Ratio between low frequency and movement frequency plus high frequency	5-minute
LF/HF ratio	Ratio between low frequency and high frequency	5-minute
Nonlinear domain[c]		
HFD	Higuchi fractal dimension quantifies the regularity of the fetal heart rate signal based on its scaling properties	5-minute
LZC	Lempel–Ziv complexity quantifies the regularity of the fetal heart rate signal converted to a sequence of symbols	5-minute
SamEn	Sample entropy measures the regularity of the fetal heart rate	5-minute
SD1	Standard deviation of the Poincaré plot perpendicular to the line of identity, which is a measure for short-term variability	5-minute
SD2	Standard deviation of the Poincaré plot along the line of identity, which is a measure for long-term variability	5-minute
SD1/SD2 ratio	Ratio between SD1 and SD2	5-minute

[a]Periods of accelerations and decelerations are excluded.
[b]The signal is first detrended using a second order polynomial.
[c]The signal is first detrended and normalized.

(HFn; 0.5–1 Hz) frequency range, the ratio between low frequency and movement frequency plus high frequency (LF/(MF + HF)), and the ratio between low frequency and high frequency (LF/HF). Nonlinear-domain analysis included the following indices: sample entropy (SamEn), the standard deviation of the Poincaré plot perpendicular to the line-of-identity (SD1), the standard deviation of the Poincaré plot along the line-of-identity (SD2), SD1/SD2, Higuchi fractal dimension (HFD), and Lempel–Ziv complexity (LZC).

FHR indices were calculated over one-minute or five-minute blocks and averaged over the duration of the FHR signal. To ensure that these blocks did not contain missing values, the signal was first divided into segments based on missing data, from which the blocks were determined. Women were subsequently excluded from further analysis if the FHR tracing did not contain at least 15 one-minute blocks or if the median baseline FHR was less than 100 bpm.

Statistical analysis

Statistical analyses were performed using R (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics were determined using descriptive statistics. Reference values for FHR were established using the Generalized Additive Models for Location Scale and Shape (GAMLSS) framework, accounting for gestational age (in days) and sex (male/female) [8]. This framework models not only the mean (location), but also the variance (scale), skewness and kurtosis (shape) of the FHR distribution. In the sub-analyses, number of fetuses (singleton/multiple), birth weight (<p10, ≥p10 and ≤ p90, >p90) [9], or time to delivery (<8 days, 8–28 days, >28days) were included as an additional explanatory variable. Appropriate model family distributions were selected based on data distribution and the family distribution with the lowest generalized Akaike information criterion was selected. The likelihood ratio test was used to assess whether including explanatory variables in the sub-analyses significantly improved model fit compared to the baseline model, with significance set at 0.05. Model fit was examined by comparing the predicted frequency to the observed frequency below selected percentiles.

Ethical approval

The study is approved by the Daily Board of the Medical Ethics Committee Erasmus Medical Center (Rotterdam, The Netherlands) (MEC-2019-0758, 2 December 2019) and was conducted in accordance with the Research Involving Human Subject Act (WMO) and the Declaration of Helsinki. The requirement for informed consent was waived in accordance with Article 24 of the GDPR Implementation Act (UAVG) and Article 458 of the Medical Treatment Contracts Act (WGBO).

Results

Of the 8902 patients who underwent FHR monitoring between July 2017 and December 2021, 5680 were excluded due to monitoring within two days of delivery, incomplete patient data, maternal medication use, insufficient FHR data, or a confirmed medical indication (Figure 1). A total of 3219 patients were included in the analysis. Patient characteristics are summarized in Table 2. The distribution of

Table 2. Patient characteristics.

	Total cohort	Male	Female
Number of patients	3219	1672 (52%)	1547 (48%)
Gestational age at first CTG	$32^{6/7}$ ($29^{1/7}$ to $36^{1/7}$)	$32^{6/7}$ ($29^{1/7}$ to $36^{1/7}$)	$32^{5/7}$ ($29^{2/7}$ to $36^{1/7}$)
Singleton (number)	2934	1537	1397
Birthweight			
<p10 (number)	729	372	357
≥p10 and ≤ p90 (number)	2210	1154	1058
>p90 (number)	280	148	132
Tracing duration (min)	42.48 (33.90–58.68)	42.49 (34.08–59.20)	42.42 (33.73–57.94)
Signal loss (%)	1.95 (0.04–8.54)	1.92 (0.04–8.46)	2.04 (0.04–8.83)

gestational ages during monitoring is shown in Figure 2. The model percentiles in weeks plus days and corresponding charts are provided in Supplement Information S1 and Figures 3–5. The parameter estimates from the GAMLSS models are presented in Table 3.

Time-domain

The mean baseline heart rate ($\beta = -8.49e^{-2}$, SE = $4.90e^{-3}$, $p < 0.001$), DPRS ($\beta = -5.22e^{-5}$, SE = $6.88e^{-8}$, $p < 0.001$), and II ($\beta = -2.52e^{-2}$, SE = $9.14e^{-4}$, $p < 0.001$), decreased significantly with advancing gestational age. In contrast, mean AAC ($\beta = 3.19e^{-3}$, SE = $5.43e^{-4}$, $p < 0.001$), ADC ($\beta = 2.78e^{-3}$, SE = $5.43e^{-4}$, $p < 0.001$), APRS ($\beta = 2.27e^{-4}$, SE = $2.06e^{-6}$, $p < 0.001$), kurtosis ($\beta = 1.05e^{-3}$, SE = $5.28e^{-4}$, $p < 0.05$), LTV($\beta = 2.01e^{-2}$, SE = $2.19e^{-3}$, $p < 0.001$), RMSSD ($\beta = 2.26e^{-3}$, SE = $3.76e^{-6}$, $p < 0.001$), and SD ($\beta = 1.01e^{-2}$, SE = $7.44e^{-4}$, $p < 0.001$) increased significantly with advancing gestational age. Only baseline heart rate showed a significant difference between sexes, with mean baseline heart rate 1.51 bpm higher in females compared to males (SE = 0.27, $p < 0.001$).

Frequency-domain

The VLFn ($\beta = -5.37e^{-3}$, SE = $3.36e^{-4}$, $p < 0.001$), decreased significantly with advancing gestational age, while LFn ($\beta = 5.38e^{-3}$, SE = $3.54e^{-4}$, $p < 0.0001$), MFn ($\beta = 5.26e^{-3}$, SE = $3.35e^{-4}$, $p < 0.001$), HFn ($\beta = 4.48e^{-3}$, SE = $2.96e^{-4}$, $p < 0.001$), and LF/HF ($\beta = 3.42e^{-2}$, SE = $1.21e^{-2}$, $p < 0.01$) increased significantly with gestational age. Females showed significantly higher values in VLFn ($\beta = 6.21e^{-2}$, SE = $1.90e^{-2}$, $p < 0.01$) compared to males, and lower values in LFn ($\beta = -6.75e^{-2}$, SE = $2.00e^{-2}$, $p < 0.001$), MFn ($\beta = -3.74e^{-2}$, SE = $1.90e^{-2}$, $p < 0.05$), LF/HF ratio ($\beta = -2.63$, SE = 0.69, $p < 0.001$), and LF/(MF + HF) ($\beta = -0.16$, SE = 0.08, $p < 0.05$).

Nonlinear-domain

Sample entropy ($\beta = 2.59e^{-3}$, SE = $2.17e^{-4}$, $p < 0.001$), HFD ($\beta = 1.11e^{-4}$, SE = $3.14e^{-5}$, $p < 0.001$), and LZC ($\beta = 1.98e^{-3}$, SE = $1.40e^{-4}$, $p < 0.001$) increased significantly with increasing gestational age. While SD1 ($\beta = -8.10e^{-5}$, SE = $1.48e^{-5}$, $p < 0.001$) and SD1/SD2 ratio ($\beta = -6.26e^{-5}$,

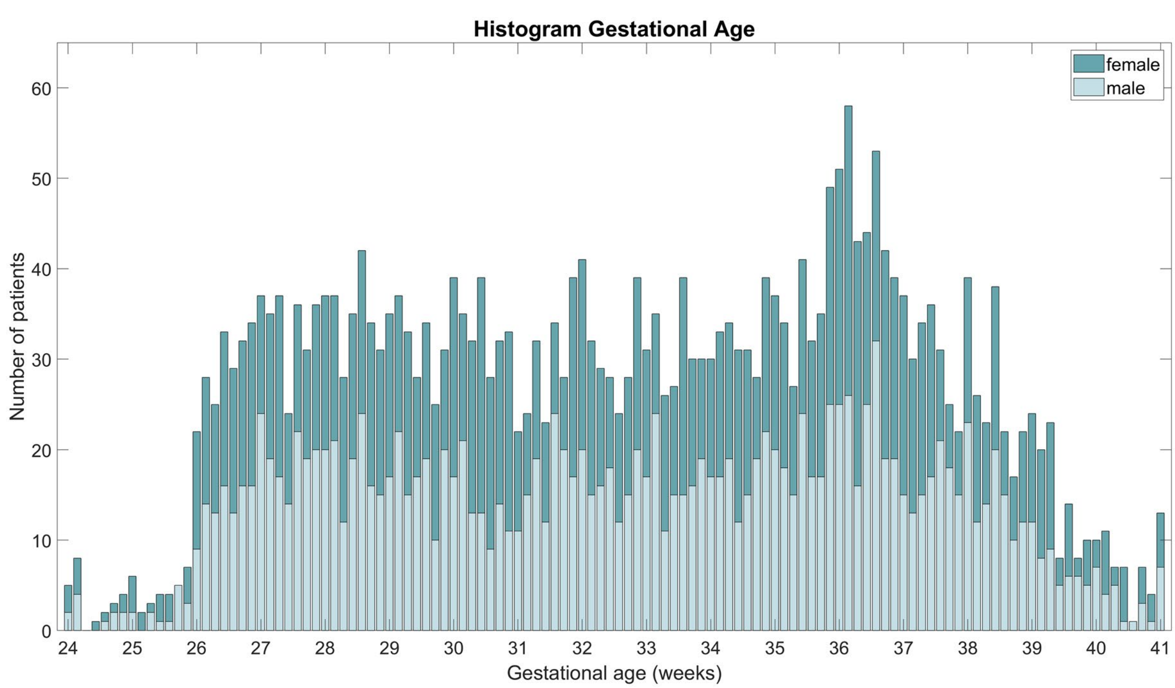

Figure 2. Histogram of the number of patients included per gestational age in weeks plus days and stacked by fetal sex.

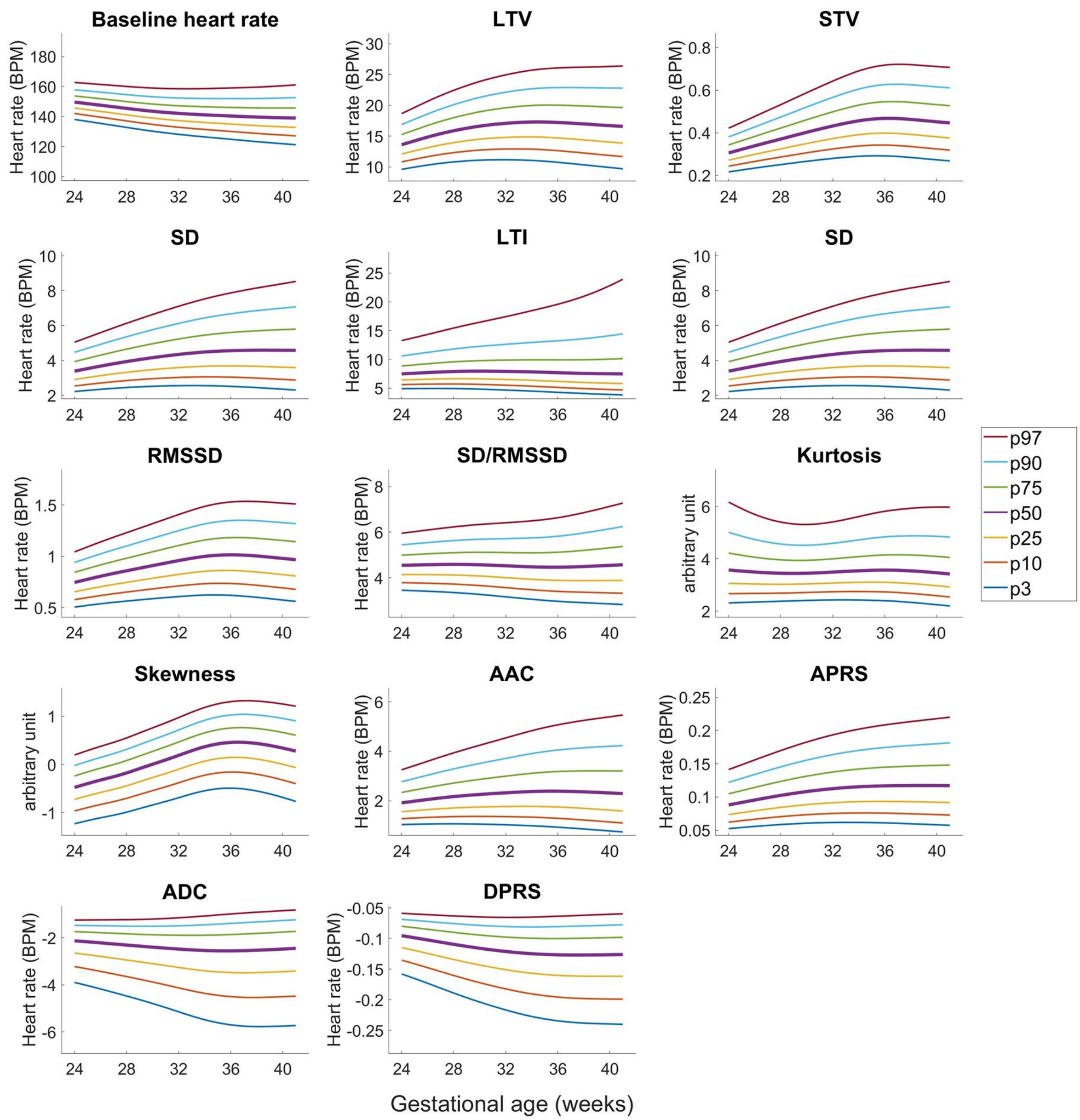

Figure 3. Reference charts for time domain heart rate indices. The chart included data of 1547 female fetuses. Abbreviations: LTV, long-term variability; II, interval index; LTI, long-term irregularity; SD, standard deviation of consecutive fetal heart rate samples; SD/RMSSD, ratio of the standard deviation and root mean square of the consecutive fetal heart rate samples; STV, short-term variability; RMSSD, root mean square of the standard deviation of consecutive fetal heart rate samples; AAC, average acceleration capacity; ADC, average deceleration capacity; APRS, acceleration phase-rectified slope; DPRS, deceleration phase-rectified slope.

SE = $1.20e^{-5}$, $p < 0.001$) significantly decreased with increasing gestational age. SD1 ($\beta = 1.82e^{-3}$, SE = $8.49e^{-4}$, $p < 0.05$) and LZC ($\beta = 1.98e^{-2}$, SE = $8.05e^{-3}$, $p < 0.05$), were significantly higher in female compared to male fetuses.

Subanalysis

The likelihood ratio test statistics are presented in Table 4. Corresponding model percentiles in weeks plus days and GAMLSS test statistics can be found in Supplement Information S2–S4. Inclusion of number of fetuses significantly improved model fit for RMSSD, skewness, MFn, HFn, LF/(MF + HF)

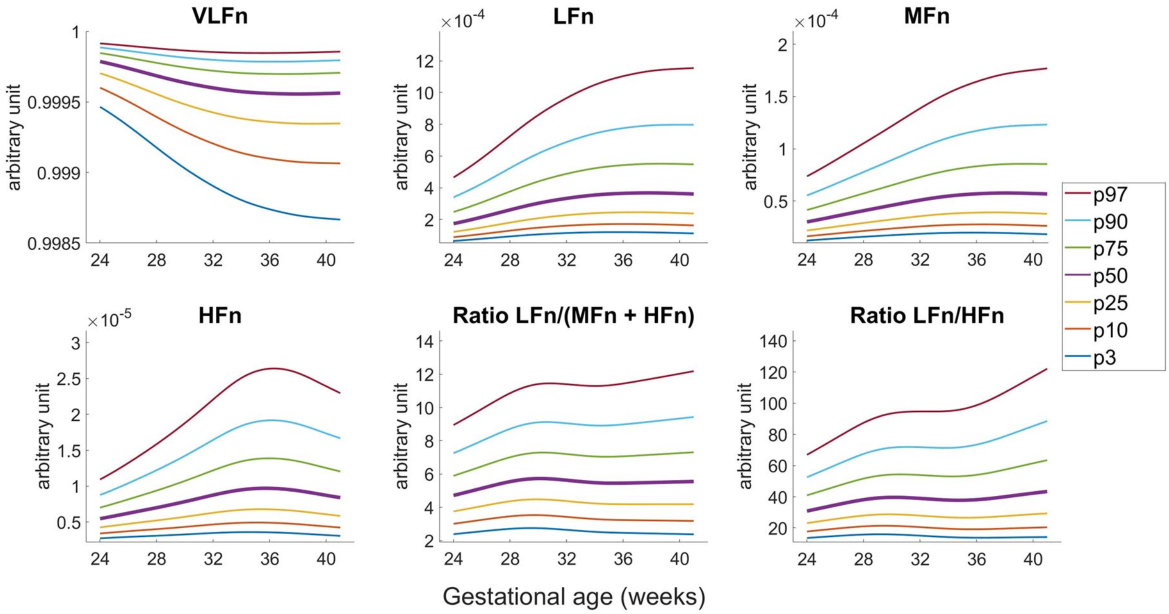

Figure 4. Reference charts for frequency domain heart rate indices of 3219 fetuses. The chart included data of 1547 female fetuses. Abbreviations: VLFn, normalized power in the very low frequency band; LFn, normalized power in the low frequency band; MFn, normalized power in the movement frequency band; HFn, normalized power in the high frequency band; LF/(MF + HF), ratio between low frequency and movement frequency plus high frequency; LF/HF, ratio between low frequency and high frequency.

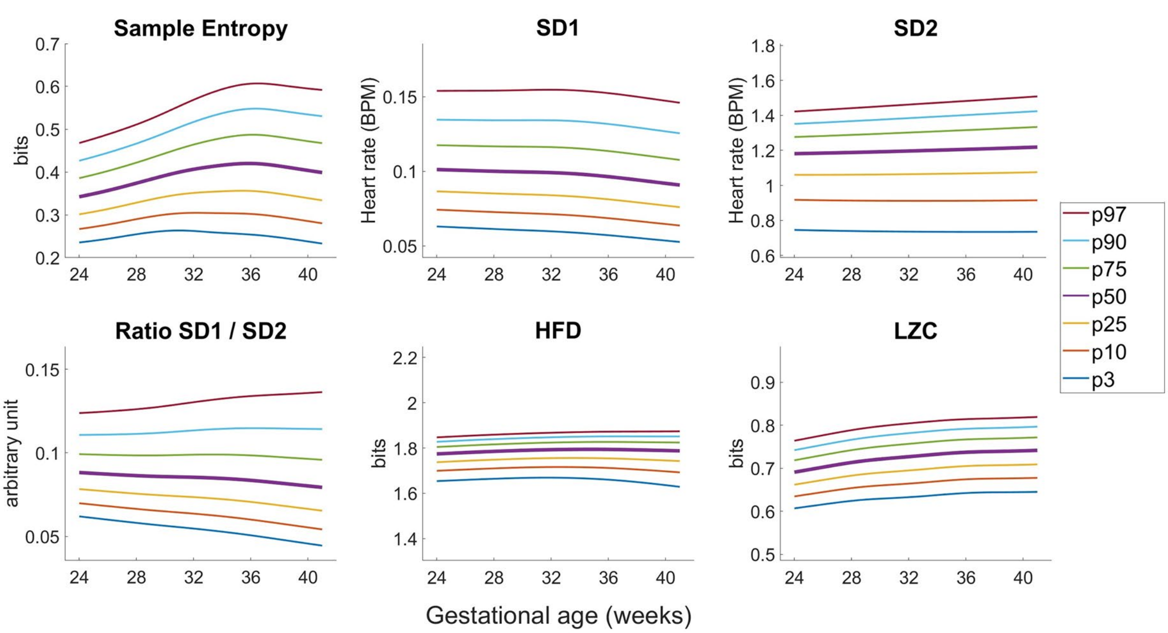

Figure 5. Reference charts for nonlinear domain heart rate indices of 3219 fetuses. The chart included data of 1547 female fetuses. Abbreviations: HFD, Higuchi fractal dimension; LZC, Lempel–Ziv complexity; SD1, standard deviation of the Poincaré plot perpendicular to the line of identity; SD2, standard deviation of the Poincaré plot along the line of identity; SD1/SD2, ratio between SD1 and SD2.

ratio, SamEn, and LZC. Including birth weight significantly improved model fit for AAC, APRS, baseline, ADC, LTV, DPRS, LTI, RMSSD, SD/RMSSD, SD, STV, VLFn, LFn, MFn, HFn, LF/HF ratio, LF/(MF + HF) ratio, SD1, SamEn, and LZC. Inclusion of time to delivery significantly improved model fit for AAC, APRS, ADC, LTV, DPRS, LTI, RMSSD, SD/RMSSD ratio, SD, VLFn, LFn, MFn, HFn, LF/HF ratio, LF/(MF + HF) ratio, SD1, and SD2.

Table 3. Parameter estimates for the location (mu) and scale (sigma) parameter in the GAMLSS model for all heart rate indices.

			Parameter estimates	
	Outcome variable	Covariate	Mu (SE)	Sigma (SE)
Time domain	AAC	Gestational age	3.19e−03 (5.43e−04)[c]	4.57e−03 (4.03e−04)[c]
		Sex	0.01 (0.03)	
	ADC	Gestational age	2.78e−03 (5.43e−04)[c]	5.64e−03 (4.96e−04)[c]
		Sex	−0.02 (0.03)	
	APRS	Gestational age	2.27e−04 (2.06e−05)[c]	2.54e−03 (4.40e−04)[c]
		Sex	−7.41e−04 (1.18e−03)	
	Baseline	Gestational age	−0.08 (4.90e−03)[c]	4.59e−03 (6.19e−04)[c]
		Sex	1.51 (0.27)[c]	
	DPRS	Gestational age	−5.22e−05 (6.88e−08)[c]	4.59e−03 (4.49e−04)[c]
		Sex	6.89e−04 (1.19e−03)	
	II	Gestational age	−0.03 (9.14e−04)[c]	3.43e−03 (7.34e−04)[c]
		Sex	−0.10 (0.05)	
	Kurtosis	Gestational age	1.05e−03 (5.28e−04)[a]	1.40e−03 (7.55e−04)
		Sex	−0.04 (0.03)	
	LTI	Gestational age	−2.53e−03 (1.69e−03)	4.61e−03 (7.20e−04)[c]
		Sex	−0.04 (0.09)	
	LTV	Gestational age	0.02 (2.19e−03)[c]	3.43e−03 (4.59e−04)[c]
		Sex	−0.15 (0.13)	
	RMSSD	Gestational age	2.25e−03 (3.76e−06)[c]	2.57e−03 (8.29e−04)[c]
		Sex	−6.41e−04 (7.24e−03)	
	SD	Gestational age	0.01 (7.44e−04)[c]	3.88e−03 (7.56e−04)[c]
		Sex	−0.03 (0.04)	
	SD/RMSSD	Gestational age	−1.23e−03 (6.31e−04)	4.64e−03 (6.37e−04)[c]
		Sex	−0.02 (0.03)	
	Skewness	Gestational age	9.47e−03 (3.18e−04)[c]	2.74e−03 (4.82e−04)[c]
		Sex	−0.01 (0.02)	
	STV	Gestational age	1.43e−03 (5.53e−05)[c]	3.08e−03 (4.59e−04)[c]
		Sex	−6.80e−04 (3.16e−03)	
Frequency domain	VLF	Gestational age	−5.37e−03 (3.36e−04)[c]	1.63e−03 (3.99e−04)[c]
		Sex	0.06213 (0.01903)[b]	
	LFn	Gestational age	5.38e−03 (3.54e−04)[c]	1.41e−03 (3.97e−04)[c]
		Sex	−0.06752 (0.02007)[c]	
	MFn	Gestational age	5.28e−03 (3.34e−04)[c]	1.99e−03 (4.00e−04)[c]
		Sex	−0.03742 (0.01895)[a]	
	HFn	Gestational age	4.48e−03 (2.96e−04)[c]	3.21e−03 (4.00e−04)[c]
		Sex	9.55e−03 (0.01706)	
	LF/(MF + HF)	Gestational age	4.57e−04 (1.39e−03)	1.78e−03 (5.83e−04)[b]
		Sex	−2.63188 (0.68795)[c]	
	LF/HF ratio	Gestational age	0.03419 (0.01211)[b]	2.52e−03 (4.03e−04)[c]
		Sex	−0.15836 (0.07579)[a]	
Nonlinear domain	HFD	Gestational age	1.11e−04 (3.14e−05)[c]	1.48e−03 (4.54e−04)[b]
		Sex	−2.19e−03 (1.63e−03)	
	LZC	Gestational age	1.98e−03 (1.40e−04)[c]	1.58e−03 (3.99e−04)[c]
		Sex	0.0198 (8.05e−03)[a]	
	SamEn	Gestational age	2.59e−03 (2.17e−04)	4.53e−03 (3.97e−04)[c]
		Sex	0.0158 (0.0122)	
	SD1	Gestational age	−8.07e−05 (1.48e−05)[c]	1.10e−03 (4.62e−04)[a]
		Sex	1.82e−03 (8.48e−04)[a]	
	SD2	Gestational age	−2.26e−04 (3.35e−04)	4.59e−03 (2.15e−03)[a]
		Sex	2.81e−03 (5.09e−03)	
	SD1/SD2	Gestational age	−6.26e−05 (1.20e−05)[c]	4.04e−03 (4.64e−04)[c]
		Sex	8.59e−04 (6.81e−04)	

Abbreviation: SE, standard error.
[a]$p < 0.05$.
[b]$p < 0.01$.
[c]$p < 0.001$.

Discussion

We established reference values for FHR indices across time, frequency, and nonlinear domain, contributing to a more comprehensive understanding of FHR variation and complexity.

Our findings reaffirmed the influence of gestational age on FHR, demonstrating developmental changes across these domains, consistent with previous studies [10–17]. Although international guidelines recommend considering gestational age when interpreting FHR patterns, fixed numerical thresholds are still commonly used for baseline heart rate. For example, for baseline heart rate, guidelines

Table 4. Likelihood ratio test for the inclusion of number of fetus (FA), birth weight percentile (BW), and time to delivery (TTD) in the GAMLSS model for all heart rate indices.

HR indices		Covariate FA	Covariate BW	Covariate TTD
		χ^2 (df)	χ^2 (df)	χ^2 (df)
Time domain	AAC	$4.31e^{-3}$ (1.00)	33.15 (3.85)[b]	28.33 (0.02)[b]
	APRS	0.34 (1.00)	39.97 (4.01)[b]	37.04 (2.01)[b]
	Baseline	1.93 (0.99)	28.21 (3.88)[b]	0.07 (1.92)
	ADC	1.17 (0.98)	45.26 (4.07)[b]	33.32 (0.74)[b]
	LTV	2.56 (1.01)	39.11 (3.91)[b]	40.43 (1.51)[b]
	DPRS	1.10 (0.99)	56.45 (4.00)[b]	39.35 (1.96)[b]
	II	1.60 (0.95)	6.14 (3.80)	4.67 (1.84)
	Kurtosis	0.63 (1.03)	0.93 (4.02)	2.19 (0.87)
	LTI	2.31 (1.06)	15.78 (3.93)[a]	8.30 (0.09)[b]
	RMSSD	4.56 (1.00)[a]	31.75 (3.94)[b]	28.19 (0.83)[b]
	SD/RMSSD	0.15 (0.98)	10.76 (4.02)	11.70 (1.23)[b]
	SD	1.76 (1.00)	39.33 (3.97)[b]	44.89 (0.95)[b]
	Skewness	4.39 (1.01)[a]	1.10 (4.00)	3.31 (1.84)
	STV	0.03 (1.02)	52.84 (4.00)[b]	29.66 (1.77)[b]
Frequency domain	VLFn	1.13 (1.01)	36.54 (3.94)[b]	43.16 (1.49)[b]
	LFn	0.67 (1.01)	38.64 (3.94)[b]	42.63 (1.54)[b]
	MFn	7.57 (1.02)[b]	15.00 (3.95)[a]	22.75 (1.38)[b]
	HFn	6.56 (0.92)[b]	22.17 (4.11)[b]	18.15 (1.74)[b]
	LF/HF	3.48 (0.95)	26.28 (3.96)[b]	16.64 (1.71)[b]
	LF/(MF + HF)	7.32 (0.91)[b]	19.67 (4.01)	11.37 (1.82)[a]
Nonlinear domain	HFD	2.87 (1.04)	8.17 (3.90)	4.15 (1.58)
	LZC	18.14 (0.77)[b]	37.66 (3.94)[b]	0.60 (1.83)
	SamEn	12.58 (0.98)[b]	19.79 (3.95)[b]	−0.02 (1.99)
	SD1	5.42 (1.00)[a]	15.09 (4.00)[a]	6.27 (2.00)[a]
	SD2	3.06 (1.00)	2.55 (4.00)	10.61 (2.00)[a]
	SD1/SD2	0.07 (1.00)	8.07 (4.00)	3.31 (2.00)

Abbreviation: df, degrees of freedom.
[a]$p < 0.05$.
[b]$p < 0.001$.

set a lower limit of 110 bpm [1]. However, our study identified higher lower limits throughout gestation; 137/138 bpm for males/females at 24 weeks of gestation, and 120/121 bpm for males/females at 41 weeks. This discrepancy suggests that current thresholds may misclassify the baseline heart rate as normal, particularly at earlier gestational ages. These observations emphasize the value of gestational age specific reference charts in daily clinical practice. Refining such charts could reduce the risk of incorrectly identifying fetal distress.

The literature reports conflicting results regarding the influence of sex on FHR indices. Several studies have reported that female fetuses show higher baseline FHR and lower variability compared to male fetuses, suggesting potential sex-related influence on autonomic cardiac regulation [11,18]. However, other studies did not observe significant sex differences on baseline or variability measures [15,19]. An exploratory twin study indicated significant sex differences across time, frequency, and nonlinear domain indices, supporting the presence of sex-related differences [20]. In line with these observations, our results also indicate that baseline FHR and variability may differ between sexes, implying that fetal sex may contribute to cardiovascular regulation during pregnancy and thus could be relevant for FHR monitoring. The underlying mechanisms may be related to differences in the hormonal environment, placental function, and maturation of parasympathetic regulation in male and female fetuses. Investigating these biological pathways alongside FHR indices further may help clarify the mechanisms underlying fetal autonomic development. Nonetheless, conventional clinical guidelines do not consider sex when interpreting FHR.

For several FHR indices, model fit significantly improved when including number of fetuses, birth weight percentile, or time to delivery, indicating measurable differences in FHR associated with these clinical factors. This suggests that underlying physiological processes related to multiple gestations, fetal growth, and preparatory preceding labor may influence FHR regulation. This is in line with previous studies that found significant differences in FHR indices between small for gestational age group fetuses and appropriate for gestational age group fetuses [14,21]. To the best of our knowledge, the influence of multiple pregnancies or time to delivery on FHR indices has not been previously studied.

FHR indices provide insights into the maturation and functioning of the autonomic nervous system. The findings of this study support that gestational age and sex should be considered when establishing FHR reference values for predictive fetal monitoring in the future. This consideration is already essential when assessing key FHR parameters in daily clinical practice. Our results showed, for example, that the interpretation of baseline heart rate should differ between a 24-week fetus and a 41-week fetus. These distinctions are vital for accurate clinical assessments and decision-making. From a clinical perspective, our findings suggest that interpretation of FHR patterns could be refined by adopting a more individualized, physiology-based approach that considers gestational age and fetal sex. Nevertheless, outcome-based validation studies are needed before any changes to clinical practice or guideline thresholds can be considered. It should be emphasized that this study does not support immediate changes to clinical guidelines, as outcome-based validation is required before any clinical implementation. Longitudinal or clinically validated prospective studies would be particularly valuable for validating the prognostic utility of the present reference values. Further research could study the influence of other clinical parameters, such as maternal medication administration or pregnancy complications, on the FHR indices and investigate their additive value for inclusion in a predictive monitoring tool. In addition, future research could study the relationship between FHR indices and clinical outcomes, with the aim of identifying discriminative factors between healthy and pathological fetal conditions. Finally, at present there is a lack of standardization of data collection, preprocessing, and analysis procedures. Future studies should prioritize addressing this issue to enhance the ease of comparing and validating study outcomes.

Strengths and limitations

The large sample size and the large number of patients included per gestational age are major strengths of this study, resulting in representative reference values for a clinical tertiary hospital population. Another strength is the extensive inclusion of fetal heart indices from the time-domain, frequency-domain, and nonlinear-domain and incorporating gestational age, fetal sex, and other clinical variables. This contributes to a more comprehensive and objective understanding of the variability and complexity of the FHR. Finally, the use of GAMLSS is a key methodological strength. GAMLSS allows flexible modeling of distributional changes across gestation, including location, variability, and shape, rather than relying on mean-based approaches alone. Taken together, these aspects distinguish the present study from previously published papers, which has predominantly relied on smaller cohorts, conventional CTG parameters, and more limited statistical approaches.

A limitation of this study is the cross-sectional design that was used to determine the reference values, as it does not address intra sample variation. It limits the ability to assess longitudinal changes within individual fetuses. In addition, the generalizability of the results may be limited due to the hospital population used to derive the reference values. Also, pregnancy outcome was not considered in the analysis.

Conclusions

This study established reference values for FHR indices across time-domain, frequency-domain, and nonlinear-domain from 24⁰ to 41⁰ weeks of gestation, derived from a tertiary hospital population and considering fetal sex. The influence of gestational age on FHR was reaffirmed. Differences based on sex, number of fetuses, birth weight percentile, and time to delivery were highlighted, although the clinical significance is not determined and needs further research. This study contributes to a more comprehensive understanding of fetal autonomic regulation by including a wide range of FHR indices and clinical parameters, and suggests that individualized predictive monitoring may offer additional clinical value. Future research should explore personalized monitoring strategies aimed to improve fetal assessment and optimize fetal care.

Author contributions

CRediT: **Chantal Eenkhoorn**: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft; **Tom G. Goos**: Conceptualization, Methodology, Writing – review & editing; **Arie Franx**: Conceptualization, Writing – review & editing; **Jenny Dankelman**: Conceptualization, Writing – review & editing; **Sten P. Willemsen**: Methodology, Writing – review & editing; **Alex J. Eggink**: Conceptualization, Writing – review & editing.

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Data availability statement

The data that support the findings of this study are available upon reasonable request.

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