Chantal Eenkhoorn*, Tom G. Goos, Arie Franx, Jenny Dankelman, H. Rob Taal, Sten P. Willemsen and Alex J. Eggink

Reference values for heart rate frequency and variability in premature neonates during the first week of life

https://doi.org/10.1515/jpm-2025-0439
Received August 12, 2025; accepted January 11, 2026;
published online February 2, 2026

Abstract

Objectives: This study aimed to establish reference values for heart rate frequency and variability indices in preterm neonates admitted to a neonatal intensive care unit of a tertiary care hospital during their first week of life.
Methods: In this retrospective cohort study, the Generalized Additive Models for Location Scale and Shape framework was employed to establish reference values for heart rate indices across time-domain, frequency-domain and nonlinear-domain in neonates considering gestational age, postnatal age, sex and birth weight.
Results: Heart rate tracings of 594 neonates (45 % female; median gestational age at birth 29^0 (IQR 26^6–30^5); 38 % birth weight <p10, 6 % birth weight >p90) were analyzed. Reference values were established for 25 heart rate indices. Nearly all heart rate indices were significantly influenced by gestational age, postnatal age and sex. Baseline heart rate decreased with gestational age, increased with postnatal age and was higher in females. Heart rate standard deviation increased with gestational age and postnatal age and was lower in female. Inclusion of birth weight significantly improved model fit for all HRV indices.
Conclusions: This study highlights the importance of considering gestational and postnatal age, sex and birth weight when interpreting neonatal heart rate frequency and variability in preterm neonates. These findings support the need for personalized approaches for neonatal monitoring and interpretation. Future research should validate these values in larger, more diverse populations, including additional clinical factors such as neonatal complications and medication administration, to determine their clinical relevance.

Keywords: neonatal heart rate variability; reference values; GAMLSS; prematurity

Introduction

In 2020, an estimated 13.4 million neonates were born preterm worldwide, a prevalence that has remained stable over the past decade [1]. Premature birth, defined as birth under 37 weeks of gestation, significantly contributes to neonatal and infant mortality, as well as long-term morbidity [2, 3]. The risk and severity of complications, including death, increase as gestational age decreases, with prematurity being a risk factor in over 50 % of all neonatal deaths [2, 3]. Neonates born prematurely and requiring intensive care are admitted to the neonatal intensive care unit (NICU). In the NICU, continuous heart rate monitoring from admission to discharge is mainly used to detect bradycardia, but also provides valuable insights into autonomic control, physiologic variability, oxygenation and blood volume of the neonate [4].

Heart rate regulation is primarily controlled by the autonomic nervous system. The interaction between the sympathetic and parasympathetic nervous systems results in beat-to-beat fluctuations that facilitates dynamic adaptation and maintenance of cardiovascular homeostasis [5]. These fluctuations, quantified as heart rate variability, reflect the autonomic nervous system's functional state and maturation. Variations in heart rate variability is influenced by physiological processes such as circadian rhythms, sleep-wake cycles, postural changes, and ongoing autonomic

***Corresponding author: Chantal Eenkhoorn**, Department of Obstetrics and Gynecology, Erasmus MC, University Medical Center, Wytemaweg 80, Rotterdam 3015CN, The Netherlands, E-mail: c.eenkhoorn@erasmusmc.nl. https://orcid.org/0000-0002-0192-2723
Tom G. Goos, Department of Neonatal and Pediatrics Intensive Care, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; and Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands
Arie Franx and Alex J. Eggink, Department of Obstetrics and Gynecology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
Jenny Dankelman, Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands
H. Rob Taal, Department of Neonatal and Pediatrics Intensive Care, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
Sten P. Willemsen, Department of Biostatistics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

maturation [6–10]. However, it can also indicate early signs of distress [11, 12]. Reduced heart rate variability has been associated with adverse outcomes, such as sepsis, necrotizing enterocolitis and adverse neurodevelopmental outcome [13–15], underscoring its potential as a valuable biomarker. Continuous monitoring of neonatal heart rate variability can detect autonomic dysregulation, revealing early signs of distress before the onset of overt symptoms of conditions such as sepsis, respiratory distress and necrotizing enterocolitis emerge. This provides clinicians with the opportunity for timely decision making and targeted intervention, such as adjusting ventilatory support, initiating medications, or closely monitoring of at-risk patients.

Reference values for heart rate can be valuable for assessing neonatal wellbeing, as deviations from these established reference values might reflect underlying distress. Multiple studies have examined heart rate variability in neonates using indices from time-domain [8, 16, 17], frequency-domain [8–10, 16–20], and the nonlinear-domain [16, 17]. These studies have investigated normative patterns while considering factors such as gestational age [8–10, 16, 19], postnatal age [9, 10, 16], sex [9], sleep state [20], sleeping position [9], and respiration [10, 19]. The majority of these studies were based on short-term recordings or were limited to the first day of life, which provides only a partial view of early autonomic development. And although reference values for heart rate frequency have been reported across gestational and postnatal ages [21], heart rate variability indices were not included in that analysis. This leaves a gap in our understanding of autonomic regulation during the early neonatal period.

In contrast to previous research, this study advances the current understanding by analyzing continuous heart rate measurements throughout the entire first postnatal week in a large cohort of preterm neonates. Through the assessment of heart rate frequency and variability indices across time-, frequency-, and nonlinear-domains, it offers a comprehensive characterization of autonomic maturation and its modulating factors during early life. The aim of this study is to establish reference values for heart rate frequency and variability indices in the time-domain, frequency-domain and nonlinear-domains in a population of neonates admitted to the NICU of a tertiary care hospital during the first week of life, born at a gestational age between 24^{+0} and 34^{+6}, taking into account sex. Furthermore, the influence of birth weight on the heart rate indices is explored.

Materials and methods

A retrospective cohort study was performed to analyze heart rate measurements during the first week of life in the NICU of a tertiary hospital (Erasmus Medical Center, Sophia Children's hospital, Rotterdam, the Netherlands). Neonates born between July 1, 2017 and December 31, 2021, with a gestational age ranging from 24^{+0} to 34^{+6} weeks, were eligible for inclusion. Exclusion criteria included out-of-hospital birth or no daily heart rate measurement available. Heart rate data, gestational age at birth, date and time of birth, sex, and birth weight were obtained from the hospital's neonatal database.

Neonatal heart rate was continuously monitored using the Infinity-M540 patient monitor (Dräger Medical, Lübeck, Germany). The Infinity-M540 calculates the neonatal heart rate by averaging the R-R intervals from the electrocardiogram over the most recent 10 s of the measurement, excluding the two longest and shortest R-R intervals [22]. It provides an averaged heart rate reading every second, with a measurement range of 15–300 beats per minute and a resolution of 1 beat per minute. In case where electrocardiography electrodes were not used, which is standard practice in neonates under 28 weeks gestational age, data from the SET pulse oximeter module (Masimo, Irvine, California, USA) connected to the M540 was utilized. The SET pulse oximeter calculates heart rate from peripheral flow pulse data, averaging the most recent 10 s of measurement and providing an output every second with a measurement range of 25–240 beats per minute and a resolution of 1 beat per minute [23]. Heart rate measurements from the Infinity-M540 and SET pulse oximeter were considered equivalent, as previous research found no statistically significant difference between average heart rates obtained from ECG and SET pulse oximeter in newborns [24].

The neonatal heart rate was continuously recorded 24 h a day, covering all behavioral states. Neonates were predominantly in the supine position, consistent with standard NICU care, although brief changes could occur during routine medical care or during skin-to-skin contact.

Heart rate data analysis was performed using MATLAB software (MATLAB 2023B, The MathWorks Inc.). Preprocessing steps were performed prior to calculating the heart rate indices: duplicate heart rate values with identical timestamps were removed, values below 30 beats per minute and above 240 beats per minute were considered artifacts and were filled as missing, and data gaps less than 20 s were filled using cubic spline interpolation, only if the heart rate value before and after the gap differed by less than 50 beats per minute.

Subsequently, heart rate indices from time-domain, frequency-domain, and nonlinear-domain were calculated. The heart rate indices and their definitions are presented in Table 1 and Supplementary Material Appendix 1. The time-domain indices included: average acceleration capacity (AAC), average deceleration capacity (ADC), acceleration phase-rectified slope (APRS), baseline, deceleration phase-

rectified slope (DPRS), interval index (II), kurtosis, long term irregularity (LTI), long term variability (LTV), median, root mean square of the standard deviation (RMSSD), standard deviation (SD), ratio between SD/RMSSD, skewness and short term variability (STV). The frequency domain indices included: normalized power in the very low frequency (VLFn; 0–0.03 Hz), low frequency (LFn; 0.03–0.15 Hz), movement frequency (MFn; 0.15–0.5 Hz) range and the ratio between low frequency and movement frequency (LF/(MF)). To calculate the frequency-domain indices a power spectral analysis was performed using a Hann window with sequences of length 256 and an overlap of 62.5 %. The nonlinear domain indices included: Higuchi fractal dimension (HFD), Lempel-Ziv complexity (LZC), 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) and ratio between SD1/SD2. The heart rate indices were calculated over one-minute, five-minute or 20 minute blocks in accordance with established standards.

Table 1: Heart rate indices definitions. Definitions of the indices across time, frequency and nonlinear domains are provided. Also the signal length over which they are calculated is given.

Index	Definition	Block length
Time domain		
AAC	Average acceleration capacity is an integral measure of all periodic acceleration-related oscillations	5-min
ADC	Average deceleration capacity is an integral measure of all periodic deceleration-related oscillations	5-min
APRS	Acceleration phase-rectified slope describes the average increase in heart rate and time length of the increase	5-min
Baseline	Baseline heart rate is the mean level of the heart rate when accelerations and decelerations are excluded	1-min
DPRS	Deceleration phase-rectified slope describes the average decrease in heart rate and time length of the decrease	5-min
II[a]	Interval index quantifies a coefficient of variation and is defined as the standard deviation of consecutive heart rate samples divided by the short term variability	1-min
Kurtosis	Kurtosis measures the peakedness of the heart rate distribution	5-min
LTI[a]	Long term irregularity quantifies the interquartile range of the distribution of the modal	5-min
LTV[a]	Long term variability quantifies the difference between the maximum and minimum heart rate value	1-min
Median	Median heart rate is the middle heart rate value when the heart rate values are arranged in ascending or descending order	1-min
RMSSD	Root mean square of the standard deviation of consecutive heart rate samples	1-min
SD/RMSSD	Ratio of the standard deviation and root mean square of the consecutive heart rate samples	1-min
SD	Standard deviation of consecutive heart rate samples	1-min
Skewness	Skewness measures the deviation of symmetry of the heart rate distribution	5-min
STV[a]	Short term variability quantifies the absolute mean FHR difference on a sample-to-sample basis	1-min
Frequency domain[b]		
VLFn	Normalized power in the very low frequency band [0–0.03]	5-min
LFn	Normalized power in the low frequency band [0.03–0.15]	5-min
MFn	Normalized power in the movement frequency band [0.15–0.5]	5-min
LF/MF	Ratio between low frequency and movement frequency	5-min
Nonlinear domain[c]		
HFD	Higuchi fractal dimension quantifies the regularity of the heart rate signal based on its scaling properties	5-min
LZC	Lempel-Ziv complexity quantifies the regularity of the heart rate signal converted to a sequence of symbols	20-min
SamEn	Sample Entropy measures the regularity of the heart rate	5-min
SD1	Standard deviation of the Poincaré plot perpendicular to the line of identity, which is a measure for short-term variability	5-min
SD2	Standard deviation of the Poincaré plot along the line of identity, which is a measure for long-term variability	5-min
SD1/SD2	Ratio between SD1 and SD2	5-min

[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.

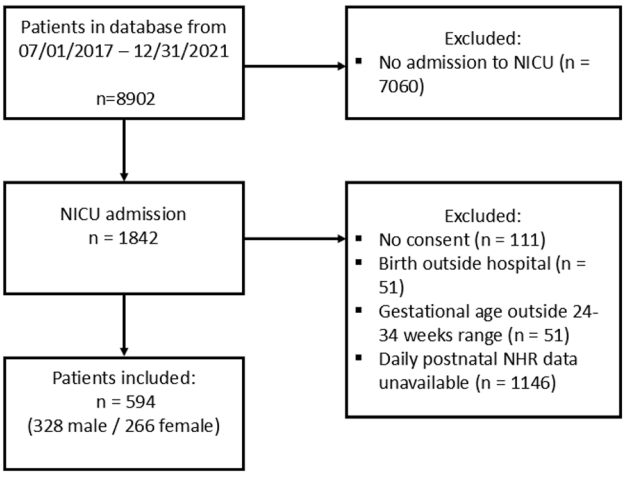

Figure 1: Flowchart of the inclusion and exclusion process for the study cohort.

Statistical analyses were performed with R (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics were determined using descriptive statistics. The Generalized Additive Models for Location Scale and Shape (GAMLSS) framework was employed to establish reference values for the heart rate, taking into account gestational age, postnatal age and sex [25]. The GAMLSS framework allows for modeling of not only the mean, but also the variance, skewness and kurtosis of the heart rate distribution. In the sub-analysis, birth weight percentile (<p10, ≥p10 & ≤p90, >p90) was added as an additional explanatory variable to the model [26]. Based on the data distribution, an appropriate model family distribution were chosen for model fitting, and the model with the lowest generalized Akaike Information Criterion was selected. The Likelihood Ratio test was used to assess whether including explanatory variable in the sub-analysis significantly improved model fit compared to the baseline model, with significance set at 0.05. The model fit was examined by comparing the predicted frequency to the observed frequency below selected percentiles.

Results

Of 1,842 newborns who were admitted to the NICU, 1,248 neonates were excluded based on predefined criteria (Figure 1). The final sample consisted of 594 neonates, of whom 45 % were female. The median gestational age at birth was 29^{+0} (IQR: 26^{+6}–30^{+5}). In this cohort, 38 % had a birth weight below the 10th percentile of the birthweight curve, whereas 6 % had a birth weight above the 90th percentile. Figure 2 illustrates the distribution of neonates according to their gestational age in full weeks at birth, categorized by sex.

Mortality occurred in 7 % of neonates, with a median age at death of 14 days (IQR 7.8–29.5). Early-onset sepsis was diagnosed in 2 %. Nasotracheal intubation was performed in 42 % of neonates, with a median age at initiation of 4.2 h (IQR 1.5–23.7) and a median duration of 2.9 days (IQR 1.1–6.5). Caffeine therapy was administered to 88 % of neonates. Vasoactive and inotropes medications were used in a subset of infants and included adrenaline (5 %), alprostadil (0.3 %), dobutamine (9 %), dopamine (3 %), fentanyl (12 %),

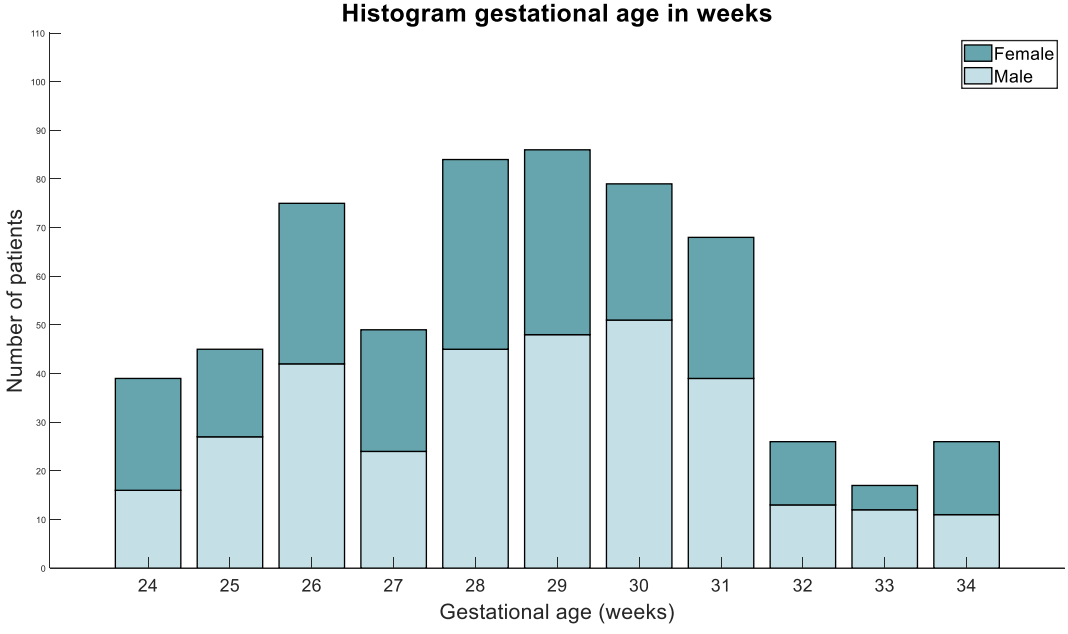

Figure 2: Histogram of the number of patients included per gestational age in weeks and stacked by sex.

isoprenaline (0.2 %), midazolam (4 %), milrinone (1 %), noradrenaline (5 %), propofol (24 %), and vasopressin (0.2 %).

Table 2 presents a summary of the GAMLSS location test statistics. The reference values and complete test statistics are provided in Supplementary Material Appendix 2. Gestational age influenced all indices except for LFn (Table 2). With advancing gestational age, mean AAC, APRS, II, LTI, LTV, RMSSD, SD/RMSSD, SD, skewness, STV, VLFn, LF/MF ratio, Sample Entropy, SD1, SD2 significantly increased. In contrast, ADC, baseline, DPRS, kurtosis, median, MFn, HFD, LZC, SD1/SD2 ratio significantly decreased. Postnatal age influenced all indices except for SD/RMSSD ratio (Table 2). With advancing postnatal age, AAC, baseline, kurtosis, LTI, LTV, Median, RMSSD, SD, STV, LFn, MFn, LF/MF ratio, HFD, LZV, Sample entropy, SD1, SD2, SD1/SD2 ratio significantly increased. The ADC, DPRS, II, skewness, VLFn significantly decreased. Male neonates exhibited significantly higher mean AAC, APRS, LTI, LTV, RMSSD, SD/RMSSD, SD, skewness, STV, LF/MF ratio, LZC, Sample Entropy, SD2. Female neonates exhibited significantly higher mean ADC, baseline, DPRS, median, MFn, SD1/SD2 ratio. Figure 3A illustrates the relation among gestational age, postnatal age and baseline heart rate for females. Figure 3B–G displays the corresponding model reference curves for the baseline heart rate for the initial 7 days after birth, categorized by the gestational age at birth in full weeks.

The likelihood ratio test statistics are presented in Table 3. Corresponding model percentiles in weeks and GAMLSS statistics can be found in Supplement Material Appendix 3. Inclusion of birth weight significantly improved model fit for all indices. Figure 4A–F illustrate the relation among gestational age, postnatal age, birth weight and baseline heart rate for

Table 2: Estimated coefficients for the location parameter (μ) in the GAMLSS model for all heart rate indices.

	Heart rate index	μ estimate (SE)			
		Intercept	Gestational age	Postnatal age	Sex
Time domain	AAC	−1.49 (0.08)[c]	0.09 (3.05e^{-3})[c]	0.05 (3.84e^{-3})[c]	−0.12 (0.01)[c]
	ADC	1.71 (0.12)[c]	−0.10 (4.86e^{-3})[c]	−0.03 (5.51e^{-3})[c]	0.07 (0.02)[c]
	APRS	−0.19 (0.01)[c]	0.01 (4.88e^{-4})[c]	8.82e^{-3} (5.85e^{-4})[c]	−0.02 (2.23e^{-3})[c]
	Baseline	212.19 (1.77)[c]	−2.32 (0.06)[c]	2.00 (0.08)[c]	0.81 (0.30)[b]
	DPRS	0.18 (0.02)[c]	−0.01 (6.10e^{-4})[c]	−4.62e^{-3} (6.99e^{-4})[c]	0.01 (2.11e^{-3})[c]
	II[a]	5.68 (0.15)[c]	0.07 (5.04e^{-3})[c]	−0.10 (5.75e^{-3})[c]	0.02 (0.02)
	Kurtosis	4.37 (0.13)[c]	−0.05 (3.95e^{-3})[c]	0.06 (3.14e^{-3})[c]	9.69e^{-5} (0.01)
	LTI[a]	−3.12 (0.29)[c]	0.31 (1.01e^{-2})[c]	0.05 (0.01)[c]	−0.40 (0.05)[c]
	LTV[a]	−8.54 (0.40)[c]	0.52 (0.01)[c]	0.20 (0.02)[c]	−0.53 (0.07)[c]
	Median	292.49 (1.75)[c]	−5.10 (0.06)[c]	1.93 (0.08)[c]	0.72 (0.30)[a]
	RMSSD	0.13 (0.04)[c]	0.01 (1.26e^{-3})[c]	0.02 (1.42e^{-3})[c]	−0.04 (5.39e^{-3})[c]
	SD/RMSSD	0.92 (0.05)[c]	0.08 (1.76e^{-3})[c]	−3.87e^{-3} (2.72e^{-3})	−0.06 (0.01)[c]
	SD	−1.39 (0.12)[c]	0.11 (4.36e^{-3})[c]	0.07 (5.29e^{-3})[c]	−0.18 (0.02)[c]
	Skewness	−0.57 (0.09)[c]	0.03 (3.15e^{-3})[c]	−0.04 (3.56e^{-3})[c]	−0.06 (9.31e^{-3})[c]
	STV[a]	−0.04 (0.02)[a]	0.01 (6.47e^{-4})[c]	1.13e^{-2} (7.95e^{-4})[c]	−0.02 (2.99e^{-3})[c]
Frequency domain	VLFn	0.97 (3.81e^{-3})[c]	4.52e^{-4} (1.30e^{-4})[c]	−4.66e^{-3} (1.60e^{-4})[c]	−4.94e^{-4} (6.11e^{-4})
	LFn	0.02 (3.39e^{-3})[c]	−1.39e^{-4} (1.16e^{-4})	4.22e^{-3} (1.43e^{-4})[c]	2.61e^{-4} (5.44e^{-4})
	MFn	9.77e^{-3} (3.94e^{-4})[c]	−2.63e^{-4} (1.31e^{-5})[c]	3.41e^{-4} (1.61e^{-5})[c]	1.33e^{-4} (6.32e^{-5})[a]
	LF/MF	−17.02 (0.45)[c]	0.84 (0.02)[c]	0.40 (0.02)[c]	−0.26 (0.08)[c]
Nonlinear domain	HFD	1.67 (0.01)[c]	−1.31e^{-3} (3.17e^{-4})[c]	8.48e^{-3} (4.11e^{-4})[c]	−3.70e^{-4} (1.87e^{-3})
	LZC	0.60 (0.01)[c]	−0.01 (4.36e^{-4})[c]	7.50e^{-3} (5.30e^{-4})[c]	−8.41e^{-3} (2.05e^{-3})[c]
	SamEn	0.29 (0.02)[c]	0.00 (5.71e^{-4})[c]	8.83e^{-3} (6.37e^{-4})[c]	−9.42e^{-3} (2.56e^{-3})[c]
	SD1	0.02 (2.60e^{-3})[c]	5.63e^{-4} (9.01e^{-5})[c]	2.64e^{-3} (1.08e^{-4})[c]	−2.42e^{-4} (4.35e^{-4})
	SD2	−0.20 (0.03)[c]	0.02 (1.10e^{-3})[c]	0.01 (1.10e^{-3})[c]	−0.02 (4.38e^{-3})[c]
	SD1/SD2	0.20 (4.43e^{-3})[c]	−3.44e^{-3} (1.49e^{-4})[c]	1.97e^{-3} (1.36e^{-4})[c]	2.27e^{-3} (5.23e^{-4})[c]

[a]p<0.05, [b]p<0.01, [c]p<0.001. AAC, average acceleration capacity; ADC, average deceleration capacity; APRS, acceleration phase-rectified slope; BW, birth weight; DPRS, deceleration phase-rectified slope; GA, gestational age; GAMLSS, generalized additive models for location scale and shape; HFD, Higuchi fractal dimension; HR, heart rate; II, interval index; LFn, normalized power in the low frequency band; LF/MF, ratio between low frequency and movement frequency; LTI, long term irregularity; LTV, long term variability; LZC, Lempel-Ziv complexity; MFn, normalized power in the movement frequency band; PNA, postnatal age; RMSSD, root mean square of the standard deviation; SamEn, Sample Entropy; SE, standard error; SD, standard deviation; SD1, standard deviation of the Poincaré plot perpendicular to the line of identity; SD1/SD2, ratio of standard deviation of the Poincaré plot perpendicular to the line of identity and standard deviation of the Poincaré plot along the line of identity; SD2, standard deviation of the Poincaré plot along the line of identity; SD/RMSSD, ratio of the standard deviation and root mean square of the standard deviation; STV, short term variability; VLFn, normalized power in the very low frequency band.

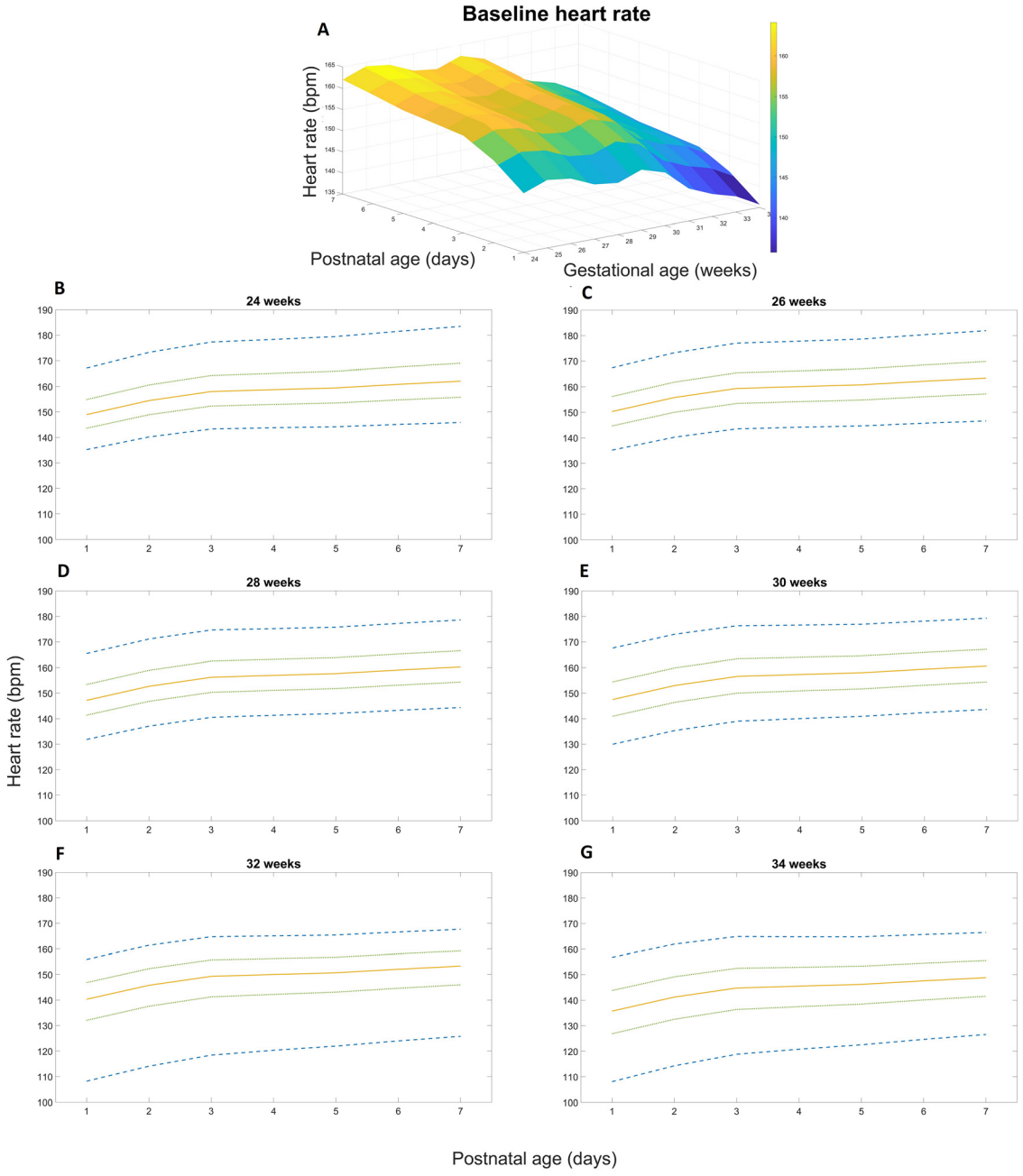

Figure 3: Graphical representation of the relationship among gestational age, postnatal age, baseline heart rate in females. (A) Mesh plot. (B–G) Model reference curves for the initial 7 days after birth, categorized by gestational age at birth in full weeks. The solid yellow line indicates the p50, the dotted green lines p25 and p75, the dashed blue lines p3 and p97. This figure displays only a subset of gestational ages (24, 26, 28, 30, 32, 34 weeks).

females and display the corresponding reference curves for the baseline heart rate for the initial 7 days after birth, categorized by the gestational age at birth in full weeks. At 24 weeks of gestational age neonates with a birth weight above the 90th percentile exhibit the highest baseline heart rate and neonates below the 10th birth weight percentile exhibit the lowest. At 34 weeks gestational age, the highest baseline heart rate postnatal is observed in neonates with a birth weight above the 10th percentile and the lowest baseline heart rate is observed in neonates with birth weight between 10th and 90th percentile.

Discussion

This study is the first to establish reference values for heart rate frequency and variability indices in the time-domain, frequency-domain and nonlinear-domain during the first

Table 3: Likelihood ratio test for the inclusion of birth weight percentile in the GAMLSS model for all heart rate indices.

Heart rate index		Covariate	
		Birth weight percentile	
		χ^2 (df)	p-Value
Time domain	AAC	115.25 (6.51)	<0.001
	ADC	44.92 (7.58)	<0.001
	APRS	139.56 (7.77)	<0.001
	Baseline	94.59 (7.81)	<0.001
	DPRS	81.55 (7.82)	<0.001
	II	22.44 (7.89)	<0.001
	Kurtosis	19.59 (7.94)	<0.001
	LTI	101.17 (8.02)	<0.001
	LTV	116.83 (8.07)	<0.001
	Median	46.36 (8.17)	<0.001
	RMSSD	201.14 (9.37)	<0.001
	SD/RMSSD	265.81 (9.40)	<0.001
	SD	78.01 (9.51)	<0.001
	Skewness	243.49 (9.75)	<0.001
	STV	164.68 (9.96)	<0.001
Frequency domain	VLFn	175.98 (9.98)	<0.05
	LFn	150.13 (9.99)	<0.01
	MFn	172.88 (10.00)	<0.001
	LF/MF	253.41 (10.35)	<0.001
Nonlinear domain	HFD	83.72 (10.53)	<0.001
	LZC	84.36 (10.54)	<0.001
	SamEn	104.67 (10.85)	<0.001
	SD1	83.55 (10.85)	<0.001
	SD2	137.87 (10.89)	<0.001
	SD1/SD2	167.12 (11.26)	<0.001

AAC, average acceleration capacity; ADC, average deceleration capacity; APRS, acceleration phase-rectified slope; BW, birth weight; DPRS, deceleration phase-rectified slope; GA, gestational age; GAMLSS, generalized additive models for location scale and shape; HFD, Higuchi fractal dimension; HR, heart rate; II, interval index; LFn, normalized power in the low frequency band; LF/MF, ratio between low frequency and movement frequency; LTI, long term irregularity; LTV, long term variability; LZC, Lempel-Ziv complexity; MFn, normalized power in the movement frequency band; PNA, postnatal age; RMSSD, root mean square of the standard deviation; SamEn, Sample Entropy; SD, standard deviation; SD1, standard deviation of the Poincaré plot perpendicular to the line of identity; SD1/SD2, ratio of standard deviation of the Poincaré plot perpendicular to the line of identity and standard deviation of the Poincaré plot along the line of identity; SD2, standard deviation of the Poincaré plot along the line of identity; SD/RMSSD, ratio of the standard deviation and root mean square of the standard deviation; STV, short term variability; VLFn, normalized power in the very low frequency band.

week of life. The research focuses on a cohort of neonates admitted to a NICU, with gestational ages between 24^{+0} to 34^{+6} weeks, and considers sex and birth weight influences. The established reference values offer significant insights into heart rate frequency and variability trends in preterm neonates during the first week of life, which could be useful for clinical monitoring in the NICU.

Similar to the observations by Cardoso et al. (2017) [6], Kiselev et al. (2024) [8], Chatow et al. (1995) [19], de Souza Filho et al. (2019) [27], Krueger et al. (2010) [28], Lavanga et al. (2021) [29] and Voss et al. (2015) [30], our results demonstrated that both gestational age and postnatal age significantly influenced nearly all heart rate variability indices across time-domain, frequency-domain, and nonlinear-domain. This consistent pattern across studies supports the notion that maturation of the autonomic nervous system is accompanied by a progressive increase in heart rate variability. The observed correlation between advancing gestational and postnatal age and enhanced heart rate variability suggests a concurrent development of both sympathetic and parasympathetic regulation during early postnatal adaptation in preterm neonates. Our findings therefore reinforce the concept of heart rate variability as a reliable marker of ANS maturation.

Sex was found to influence all indices with the exception of II, kurtosis, VLFn, LFn, HFD and SD1. The observation of sex-related differences in heart rate variability, particularly in the time-domain, reflects underlying physiological distinctions between males and females. This finding aligns with previous research that identified sex differences in heart rate among fetuses, neonates and older pediatric populations [28, 30–32], with females exhibiting higher heart rates than males. However, Cabal et al. (1980) did not identify significant sex differences [33], which may be attributable to limitations in sample size or methodological differences. The appearance of the sex differences so early in life suggest that these differences are not merely the result of postnatal development, but may be rooted in fundamental physiological processes.

Birth weight influenced all HRV indices, suggestion that birth weight plays a role in the autonomic regulation and development processes of the newborn. These findings are consistent with a previous study by Rakow et al. (2013) [34], that demonstrated an association between low birth weight and reduced heart rate variability in children, indicating that autonomic control may be adversely affected by low birth weight. In contrast to our findings, the study by Tveiten et al. (2020) [32] did not observe a significant influence of birth weight on heart rate. This discrepancy may be explained by the inclusion of primarily term neonates in their study, in which the effect of birth weight on autonomic regulation may be less pronounced compared with preterm populations.

The unique strength of this study lies in the comprehensive nature of the heart rate variability analysis, including multiple domains (time, frequency, and nonlinear) within a vulnerable population, namely preterm neonates. By providing reference values for heart rate frequency and

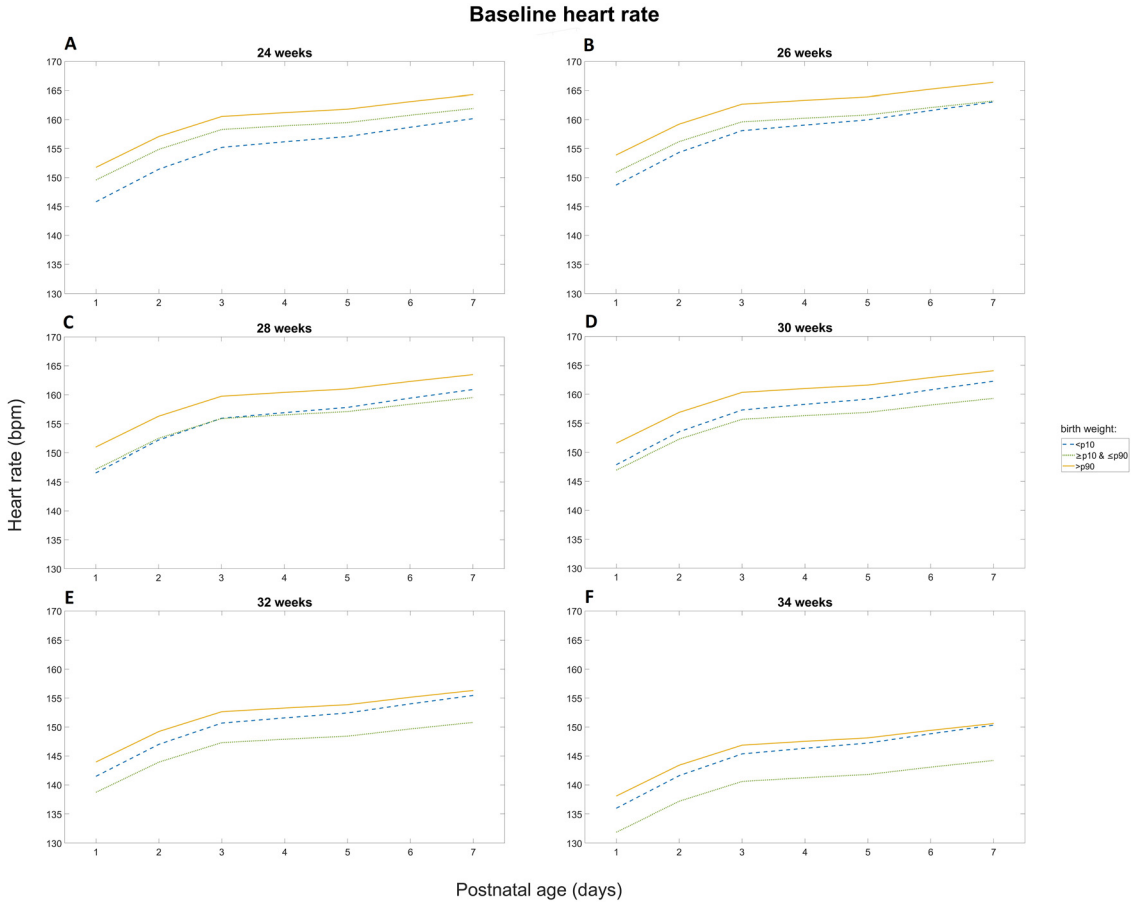

Figure 4: Graphical representation of the relationship among gestational age, postnatal age, birth weight, and baseline heart rate in females. (A–F) Model reference curves for the initial 7 days after birth, categorized by gestational age at birth in full weeks. The solid yellow line indicates the p50 for birth weight >p90, the dotted green line indicates the p50 for birth weight ≥p10 & ≤p90, the dashed blue line indicates the p50 for birth weight >p90. This figure displays only a subset of gestational ages (24, 26, 28, 30, 32, 34 weeks).

variability indices during the critical first week of life, clinicians can gain deeper insights into autonomic function and maturation in preterm infants. This output data has the potential to guide early diagnosis and treatment strategies for neonates at risk of cardiovascular instability or developmental delays. Moreover, this study emphasized the importance of considering multiple factors, gestational age, postnatal age, sex, and birth weight, when interpreting heart rate data in neonates. The observation that these factors significantly affect heart rate frequency and variability indices underscores the need for personalized approaches to neonatal monitoring. These findings highlight the potential to integrate this data into automated systems for signal and trend analysis of neonatal heart rate, enabling early warning systems to detect abnormalities in real-time and predict clinical deterioration enabling timely intervention. These physiological variables can have a considerable impact on the interpretation of heart rate variability as a clinical tool. In addition, heart rate variability indices may hold potential as biomarkers for evaluating neonatal health and predicting long-term outcomes related to autonomic nervous system function and development. The study also has limitations. The inclusion of a population admitted to the NICU could have affected the generalizability of the findings, as predictive models may be more accurate when they include a control group tailored to gestational age, postnatal age and sex. Additionally, clinical factors such as neonatal complications and medication administration were not taken into account, which could have influenced heart rate variability outcomes. Also, the clinical relevance of the found differences has not yet been studied.

Overall, this study provides new insights into the early postnatal maturation of the autonomic nervous system in preterm neonates. By integrating time-domain, frequency-domain, and nonlinear-domain analyses, we identified distinct influences of gestational age, postnatal age, sex, and birth weight on heart rate variability. These findings emphasize that multiple clinical factors must be considered when interpreting

neonatal heart rate dynamics, underscoring the potential of heart rate variability indices as sensitive markers of early autonomic function and clinical stability.

Conclusions

This study established reference values for heart rate frequency and variability indices in preterm neonates during their first week of life, with particular attention to the influence of gestational age, postnatal age, sex, and birth weight. These findings enhance our understanding of early autonomic development in neonates and support the use of heart rate variability as a clinical tool for monitoring neonatal health. Future research should focus on validating these reference values in larger and more diverse populations and exploring the potential of heart rate variability as a predictive biomarker for long-term outcomes in preterm infants. The influence of sex, birth weight and other clinical factors on heart rate variability during early life warrants further investigation, particularly in relation to its potential implications for individualized neonatal care.

Acknowledgments: The authors would like to thank Annelies Ham for her assistance with data collection.

Research ethics: The Medical Ethics Committee of Erasmus Medical Center (Rotterdam, the Netherlands) approved the study protocol (MEC-2019-0758, 2 December 2019), in accordance with the Research Involving Human Subject Act (WMO).

Informed consent: A waiver for parental informed consent was given based on the observational nature of the study.

Author contributions: C.E., T.G.G., A.J.E., designed and set up the study. C.E., T.G.G, S.P.W. determined the statistical design, C.E. collected and analyzed the data. C.E., T.G.G, A.J.E, R.T., interpreted results. C.E. wrote the manuscript. All authors edited, revised the paper and approved the final version. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools: None declared.

Conflict of interest: The authors state no conflict of interest.

Research funding: This research is part of the MOMETA program of the Medical Delta Institute of Fetal and Neonatal Care and funded by the Medical Delta Call 2.0.

Data availability: All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

References

1. Ohuma EO, Moller A-B, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. Lancet 2023;402:1261–71.
2. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health 2013;10:1–14.
3. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 2008;371:261–9.
4. Kumar N, Akangire G, Sullivan B, Fairchild K, Sampath V. Continuous vital sign analysis for predicting and preventing neonatal diseases in the twenty-first century: big data to the forefront. Pediatr Res 2020;87:210–20.
5. Kamath MV, Watanabe M, Upton A. Heart rate variability (HRV) signal analysis: clinical applications. Boca Raton, FL, USA: CRC Press; 2016.
6. Cardoso S, Silva MJ, Guimarães H. Autonomic nervous system in newborns: a review based on heart rate variability. Childs Nerv Syst 2017;33:1053–63.
7. Mirmiran M, Maas YGH, Ariagno RL. Development of fetal and neonatal sleep and circadian rhythms. Sleep Med Rev 2003;7:321–34.
8. Kiselev AR, Mureeva EN, Skazkina VV, Panina OS, Karavaev AS, Chernenkov YV. Full-term and preterm newborns differ more significantly in photoplethysmographic waveform variability than heart rate variability. Life 2024;14:675.
9. Fister P, Nolimal M, Lenasi H, Klemenc M. The effect of sleeping position on heart rate variability in newborns. BMC Pediatr 2020;20:156.
10. Van Ravenswaaij-Arts C, Hopman J, Kollee L, Stoelinga G, Van Geijn H. Spectral analysis of heart rate variability in spontaneously breathing very preterm infants. Acta Paediatr 1994;83:473–80.
11. Hashiguchi K, Kuriyama N, Koyama T, Matsui D, Ozaki E, Hasegawa T, et al. Validity of stress assessment using heart-rate variability in newborns. Pediatr Int 2020;62:694–700.
12. Gardner FC, Adkins CS, Hart SE, Travagli RA, Doheny KK. Preterm stress behaviors, autonomic indices, and maternal perceptions of infant colic. Adv Neonatal Care 2018;18:49–57.
13. Bohanon FJ, Mrazek AA, Shabana MT, Mims S, Radhakrishnan GL, Kramer GC, et al. Heart rate variability analysis is more sensitive at identifying neonatal sepsis than conventional vital signs. Am J Surg 2015;210:661–7.
14. Stone ML, Tatum PM, Weitkamp J-H, Mukherjee AB, Attridge J, McGahren ED, et al. Abnormal heart rate characteristics before clinical diagnosis of necrotizing enterocolitis. J Perinatol 2013;33:847–50.
15. Addison K, Griffin MP, Moorman JR, Lake DE, O'Shea TM. Heart rate characteristics and neurodevelopmental outcome in very low birth weight infants. J Perinatol 2009;29:750–6.
16. Oliveira V, Von Rosenberg W, Montaldo P, Adjei T, Mendoza J, Shivamurthappa V, et al. Early postnatal heart rate variability in healthy newborn infants. Front Physiol 2019;10:922.
17. Mehta SK, Super DM, Connuck D, Salvator A, Singer L, Fradley LG, et al. Heart rate variability in healthy newborn infants. Am J Cardiol 2002;89:50–3.
18. Massin MM, Maeyns K, Withofs N, Ravet F, Gérard P. Circadian rhythm of heart rate and heart rate variability. Arch Dis Child 2000;83:179–82.
19. Chatow UDI, Davidson S, Reichman BL, Akselrod S. Development and maturation of the autonomic nervous system in premature and full-term infants using spectral analysis of heart rate fluctuations. Pediatr Res 1995;37:294–302.

20. Doyle OM, Korotchikova I, Lightbody G, Marnane W, Kerins D, Boylan GB. Heart rate variability during sleep in healthy term newborns in the early postnatal period. Physiol Meas 2009;30:847.
21. Alonzo CJ, Nagraj VP, Zschaebitz JV, Lake DE, Moorman JR, Spaeder MC. Heart rate ranges in premature neonates using high resolution physiologic data. J Perinatol 2018;38:1242–5.
22. Dräger Medical AG & Co. KG. Infinity Acute Care System – Infinity M540: Instructions for Use (software VG7.n). Lübeck, Germany; 2017. [Accessed 02 Jan 2025].
23. Masimo Corporation. Radical-7 Pulse CO-Oximeter: Operator's Manual. Irvine, CA 2020.
24. Dawson J, Saraswat A, Simionato L, Thio M, Kamlin C, Owen L, et al. Comparison of heart rate and oxygen saturation measurements from Masimo and Nellcor pulse oximeters in newly born term infants. Acta Paediatr 2013;102:955–60.
25. Stasinopoulos DM, Rigby RA. Generalized additive models for location scale and shape (GAMLSS) in R. J Stat Software 2008;23:1–46.
26. Hoftiezer L, Hof MHP, Dijs-Elsinga J, Hogeveen M, Hukkelhoven C, van Lingen RA. From population reference to national standard: new and improved birthweight charts. Am J Obstet Gynecol 2019;220: 383.e1–17.
27. de Souza Filho LFM, de Oliveira JCM, Ribeiro MKA, Moura MC, Fernandes ND, de Sousa RD, et al. Evaluation of the autonomic nervous system by analysis of heart rate variability in the preterm infants. BMC Cardiovasc Disord 2019;19:1–6.
28. Krueger C, van Oostrom JH, Shuster J. A longitudinal description of heart rate variability in 28—34-week-old preterm infants. Biol Res Nurs 2010;11:261–8.
29. Lavanga M, Heremans E, Moeyersons J, Bollen B, Jansen K, Ortibus E, et al. Maturation of the autonomic nervous system in premature infants: estimating development based on heart-rate variability analysis. Front Physiol 2021;11:581250.
30. Voss A, Schroeder R, Heitmann A, Peters A, Perz S. Short-term heart rate variability—influence of gender and age in healthy subjects. PLoS One 2015;10:e0118308.
31. Bhide A, Acharya G. Sex differences in fetal heart rate and variability assessed by antenatal computerized cardiotocography. Acta Obstet Gynecol Scand 2018;97:1486–90.
32. Tveiten L, Diep LM, Halvorsen T, Markestad T. Heart rate during the first 24 hours in term-born infants. Arch Dis Child Fetal Neonatal Ed 2021; 106:489–93.
33. Cabal LA, Siassi B, Zanini B, Hodgman JE, Hon EE. Factors affecting heart rate variability in preterm infants. Pediatrics 1980;65:50–6.
34. Rakow A, Katz-Salamon M, Ericson M, Edner A, Vanpée M. Decreased heart rate variability in children born with low birth weight. Pediatr Res 2013;74:339–43.

Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/jpm-2025-0439).