Heart rate variability categories of fluctuation amplitude and complexity – diagnostic markers of fetal development and its disturbances.

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Novelty & Significance *Please state, in under 100 words, the novelty and significance of your article.*

Fetal development and its disturbances have been reported to be associated with a multiplicity of HRV indices. Furthermore, these HRV indices change with maturation. We propose the abstraction of HRV categories defined by short- and long-term fluctuation amplitude, complexity, and pattern indices that cover all relevant aspects of maturational age, behavioral influences and a series of pathological disturbances. The study data are provided by multiple centers. Our approach is an important step towards the goal of a standardized diagnostic tool for early identification of fetal developmental disturbances with respect to the reduction of serious complications in the later life.

Abstract

Objective:

In fetal diagnosis the myriad and diversity of heart rate variability (HRV) indices prevents a comparable routine evaluation of disturbances in fetal development and well-being. The work aims at the extraction of a small set of HRV key indices that could help to establish a universal, overarching tool to screen for any disturbance.

Approach:

HRV indices were organized in categories of short-term (s) and long-term (l) amplitude fluctuations (AMP), complexity (COMP), and patterns (PATTERN) and common representatives for each category were extracted. This procedure was done with respect to the diagnostic value in the evaluation of the maturation age throughout the second and complete third trimester of pregnancy as well as to potential differences associated with maternal life-style factors (physical exercise, smoking), nutrient intervention (docosahexaenoic acid (DHA) supplementation), and complications of pregnancy (gestational diabetes mellitus (GDM), intra-uterine growth restriction (IUGR)).

Main results:

We found a comprehensive minimal set that includes [lAMP: STV (initially introduced as short term variation in CTG), sAMP: ACst1 (Acceleration capacity related), lCOMP: MSE4 (scale 4 multi-scale entropy), PATTERN: skewness] for the maturation age prediction and partly overlapping with [lAMP: STV, sAMP: ACst1, sCOMP: LZC (Lempel Ziv complexity)] for the discrimination of the deviations.

Significance:

The minimal set of category-based HRV representatives allows for a screening of fetal development and well-being. These results are an important step towards a universal and comparable diagnostic tool for the early identification of developmental disturbances.

Introduction

The focus *biological oscillations and health* addresses the essential role of fluctuations in the complex dynamic behavior of an organism. The formation of these fluctuations is an essential part of the phylogenetic evolution process and remains inherent in the ontogenetic maturation process (Haeckel, 1866; Hoyer *et al.*, 2013b). Fetal developmental problems have irreversible implications for all of later life (Barker, 1998; Van den Bergh *et al.*, 2017), but their early identification remains inadequate. In that context, characteristics of fetal heart rate patterns provide a unique diagnostic window during the second and third trimester of pregnancy (Nijhuis *et al.*, 1982; Pillai and James, 1990; FIGO, 2011). Various studies have addressed heart rate variability (HRV) indices of normal physiological fetal maturation as well as developmental disturbances e.g. (Van Leeuwen *et al.*, 1999; David *et al.*, 2007; Ferrario *et al.*, 2009; Amorim-Costa *et al.*, 2017a; Amorim-Costa *et al.*, 2017b). Their results are generally consistent, but the dispersion of the results is high and the HRV indices used are diverse. This is partly caused by the variety of HRV indices available and simplified models with respect to the two (or more) influencing factors such as gestational age (GA) and physiological alterations. The sympathetic and parasympathetic branches of the autonomic nervous system (ANS) develop and mature at different rates. Consequently, developmental progression of fetal cardiac autonomic control can be measured by different HRV indices that reflect critical periods of ANS development across gestation (Schneider *et al.*, 2018). These developmental changes cannot be reflected by linear models which assume a constant development across the total second and third trimester. Furthermore, the diversity of HRV indices suggests high accuracy, but it rather leads to statistically over-fitted models and physiological over-interpretations. Based on the redundancy of the multiplicity of HRV indices, identifying a few relevant key parameters would help to propose a feasible, universally manageable and standardized diagnostic tool for early identification of fetal developmental disturbances with a view to the reduction of serious complications in later life.

The myriad different HRV indices applied in individual studies hinders easy and reliable comparisons of methods and results. Some of the indices are redundant, and others discriminative only with respect to particular influences or disturbances (TaskForce, 1992; Stein, 2005; Maestri *et al.*, 2007; Schmidt *et al.*, 2018b). Within categories of HRV amplitude, complexity, and heart rate patterns, respectively, we expect certain redundancies between the category members. It is our intention to introduce and evaluate a concept that significantly reduces the number of HRV indices without losing the option of integrating novel indices while remaining appropriate for the identification of additional, not yet considered alterations of autonomic control.

The aim of the present work is to identify key HRV categories that reflect physiological development of the fetal ANS with respect to important maturational periods during the second and third trimester. The diagnostic value of these categories is explored using several examples of maternal and fetal factors that have previously been shown to influence fetal HRV. Representatives of these key HRV categories are proposed as universal, overarching candidates for fetal screening and could be an important step towards a standardized diagnostic tool.

Methods

Subjects and recordings

From the recordings provided by the following centers, only sections of active sleep (2F) lasting at least 7 min, generally 10 min were analysed, overview see Table 1. Only the 5 min data sets from Bochum were analysed regardless of state, as they are too short for state selection.

Normal group: MCG recordings lasting 30 min of the Jena study data base (Biomagnetic Center/Department of Neurology, Department of Obstetrics/Division of Prenatal Diagnostics and Fetal Physiology, Jena University Hospital). For methodological details see e.g. (Hoyer *et al.*, 2013b).

Normal reference group: MCG recordings lasting 5 min of the Bochum study data base (Grönemeyer Institute for Microtherapy, University Witten/Herdecke, Bochum). For methodological details see (Hoyer *et al.*, 2015).

Effect of maternal exercise: Healthy women with low-risk, singleton pregnancies gave informed consent and were enrolled in a study designed to determine the effect of maternal physical activity on fetal cardiac autonomic control. Women were categorized into exercise or control groups based on self-report to a standardized questionnaire. MCG recordings lasting 18 min were recorded at 24, 32 and36 WGA (Hoglund Brain Imaging Center, Department of Neurology, University of Kansas Medical Center, Kansas City, Kansas, USA). Twenty-one women were assigned to the exercise group and 19 women to the control group. Differences in fetal HRV were found only at 36 WGA, therefore, this analysis is limited to fetal data recorded at that time point. (For details of the parent study, see (May *et al.*, 2010), Data used in this report were obtained from available public data (Van Leeuwen *et al.*, 2014a).

Effect of maternal smoking: Healthy pregnant women (n=24) with low-risk, singleton pregnancies who reported smoking during pregnancy gave informed consent and enrolled in a study designed to measure the effect of maternal smoking on fetal HRV. Women abstained from smoking overnight. MCGs of 18 minutes duration were recorded prior to and immediately after smoking their first cigarette of the day (pre- vs. post-smoking) at 24, 28, 32, 34, 36 and 38 WGA. (Hoglund Brain Imaging Center, Department of Neurology, University of Kansas Medical Center, Kansas City, Kansas, USA)

Influence of maternal docosahexaenoic acid (DHA) supplementation: Following informed consent, healthy pregnant women (n=67) with low-risk, singleton pregnancies enrolled in a randomized clinical trial (NCT01007110) designed to test the effect of DHA supplementation on fetal cardiac autonomic control. Women consumed daily capsules containing a mixture of corn/soy oil (placebo) or 600 mg of DHA during the last two trimesters of pregnancy. MCGs of 18 minutes duration were recorded at 24, 32 and 36 weeks WGA at the Hoglund Brain Imaging Center, Department of Neurology, University of Kansas Medical Center, Kansas City, Kansas, USA. For methodological details of the parent trial see (Gustafson *et al.*, 2013). Significant differences in fetal HRV were reported at 32 and 36 WGA. For this report, we used data from 32 WGA (24 placebo; 23 DHA) and 36 WGA (19 placebo; 21 DHA).

Influence of IUGR: MCG recordings from IUGR fetuses (estimated weight <10th percentile with respect to GA, pathological uteroplacental perfusion > 24 WGA) versus normal group form the Jena study data base.

Influence of IUGR reference group 1: MCG recordings from the Bochum data base and respective normal controls. For details see (Hoyer *et al.*, 2015).

Influence of IUGR reference group 2: Fetal non-invasive ECG recordings obtained from maternal abdominal surface were performed in the Kharkiv perinatal center. The methodology has been previously described (Hoyer *et al.*, 2017). Since the extraction of high-quality beat-to-beat variability using fetal ECG is still a challenge (see e.g. (van Leeuwen *et al.*, 2014b)), a total quantity of performed records were twice higher. Tracings with sufficient quality were found predominately in the periods from 20 to 28 weeks and from 34 to 36 weeks of gestation. Therefore, this peculiarity could have an influence on the fetal gestational age distribution in fetal ECG cohort.

Influence of gestational diabetes mellitus (GDM): GDM is defined by increased glucose levels during an oral glucose tolerance test OGTT, (500ml drink containing 75gr sugar). We recorded data in 13 pregnant women with GDM and 36 pregnant women with normal glucose tolerance. The participants were recorded three times, directly before intake of the solution and 60 and 120 minutes after ingestion of the glucose (For methodological details see (Fehlert *et al.*, 2017; Cysarz *et al.*, 2013).

Table 1: Study centers and data

<INSERT TABLE 1>

Inclusion criteria

Fetal heart rate recording in sinus rhythm of singletons during the second and third trimester, maternal age > 18 y, approval from the associated local Ethics Committees and written consent of the participants.
Gestational ages were calculated from the date of the last menstrual period and confirmed by first trimester crown-rump-length measurement.

Exclusion criteria (normal groups and controls in clinical cohorts)

Maternal: known heart disease, medication affecting cardiac function, smoking, abuse of alcohol or illicit drugs, diabetes mellitus (both pre-pregnancy Type I/II and gestational diabetes).

Fetal: known chromosomal abnormalities, sonographically identified malformations, uterine contractions during recording, cardiac arrhythmias, IUGR (normal groups), previous exposure to synthetic steroids for premature induction of lung maturation.

Fetal behavioral states

The fetal behavioral state is a fundamental factor of autonomic activity and HRV. In order to keep the present work clear, we report only results during active fetal sleep (2F) according to CTG standards that propose to use an active state in order to exclude adverse behavior or developmental disturbances (FIGO, 2011). The states of all data sets were classified from visual inspection of the heart rate pattern printout after a consensus decision by three independent obstetricians of the Jena study center.

HRV indices and categories

The HRV indices used include the most common ones and were organized according to their signal processing origin into categories of amplitude, complexity and patterns (Table 2). All HRV indices were calculated from preprocessed NN interval series after removal of artifacts and decelerations according to previous analyses (Hoyer *et al.*, 2014; Schmidt *et al.*, 2018a). Recordings were considered only if less than 5 % of their time length had been corrupted by artifacts. The distributions of the HRV indices were tested for normality via the Shapiro Wilk test and the visual inspection of the QQ-plot. In order to ensure a normal distribution of the HRV indices, different transformations functions (sqrt/log) were used. The signal analysis was done using MatLab2014a.

Categories of HRV indices were established with respect to their physiologic and system-theoretic origin and their correlations in the normal group. According to the linear nature of the amplitude indices we proposed a short-term category which is predominantly related to vagal activity and a long-term category that reflects both, vagal, sympathetic and humoral modulations. Complexity is a nonlinear property across less clearly separable time scales. We included all complexity indices in one category, however provided a short-term and a long-term index where appropriate. The category of patterns contains different approaches like skewness and power-ratios. Furthermore, for each multi-scale temporal asymmetry index a short-term and a long-term proxy were considered (overview see Table 2).

Table 2: HRV categories of short and long term amplitude/magnitude “AMP”, complexity “COMP”, and different patterns “PATTERN”. "l" and "s" are indicators for long term and short term versions (as used here for AMP, but also for other categories below).

<INSERT TABLE 2>

Statistical Analysis

The gestational age range was divided into overlapping age windows, namely: range 23 (20-26), range 26 (23-29), range 29 (26-32), range 32 (29-35), and range 35 (32-38) WGA. The dependency between each of the HRV category representatives and the chronological fetal age in each window was estimated by linear regression models (standardized beta coefficient beta, 95 % CI). The partial Spearman's rank correlation coefficients between the HRV indices of the Jena normal group (2F) were calculated partialized to GA in order to remove the confounding effect of GA.

The GA dependencies/prediction were compared by linear regression models, quantified by the mean standard error (SE) and coefficient of determination R^2 in a 3 fold cross-validation-scheme with 10 repeats . The SE's from the single folds are tested via Wilcoxon signed rank sum test for significant differences. The groups were compared by logistic regression models, quantified by the mean area under the curves (AUC) value of the receiver operating characteristic (ROC) in a 3 fold cross-validation-scheme with 10 repeats. The AUC's from the single folds are tested via Wilcoxon signed rank sum test for significant differences. The further group comparisons were done using Wilcoxon-Mann-Whitney U Test and the paired groups by Wilcoxon signed rank test (R version 3.3.2.). OGTT was analyzed by two-factorial variance analysis of repeated measures (IBM SPSS statistics 25). The significance level alpha was set to 0.05

Investigation flow

1. Representatives of categories with respect to normal fetal aging
    a. Validation of categories by correlation matrix of the Jena normal group.
    b. Select a representative (best age predictor) HRV index for each category.
    c. Check for synergism by investigation the best couple of HRV indices (age prediction).
2. Representatives of categories with respect to identification of different deviations (see 4.b)
    a. Evaluation of the discriminatory value of the representatives.
    (comparison of age representatives from 1b with best discriminators and the best discriminating couple of HRV indices separately for the particular deviations).
    b. Select a set of representatives with discriminatory value across all kind of deviations in comparison with the best ones of each particular deviation.
3. Propose a minimal set of HRV key indices for screening of autonomic maturation age and all kinds of deviations.
4. Application of minimal set
    a. Assessment of maturation age across 2d and 3d trimester
    b. Identification of deviations due to maternal life-style factors (physical exercise, smoking), nutrient intervention (docosahexaenoic acid (DHA) supplementation), and complications of pregnancy (gestational diabetes mellitus (GDM), intra-uterine growth restriction (IUGR)).

Results

Representatives of categories with respect to normal fetal aging

The correlation matrix (Figure 1) mainly confirms the proposed category members. mHR shows weak correlations with Parameters of lAMP and sAMP. In lAMP (AMP, SD, STV, LTV, TP, VLF, LF, ACsl4,ACst4, DCsl4,DCst4) and sAMP (pNN5, RM, P0V, P1V, P2V, HF, ACsl1,ACst1 DCsl1,DCst1), respectively, all members were clearly correlated with correlation coefficients r of about 0.7 to 1.0. In the lAmp category, the lowest correlation coefficient r = 0.55 was observed between Amp and Dcsl4 and in the sAmp category, the lowest correlation was found between P1V and ACst1 (r = 0.46). Herein, all PRSA related indices were mutually correlated as well as with sAMP and lAMP members (r = 0.6-0.9). Weaker correlations (r ≈ 0.5) could be observed between sAMP and lAMP members. Concerning short scale members of the COMP category LZC and MSE1 were not correlated. Overall, LZC shows negligible correlations with all other parameters. However, MSE1 and α1 were correlated (r = 0.76) as short scale members as well as MSE4 and α2 (r = 0.79) as long scale members. Furthermore, MSE1 and α1, respectively, show correlations (r ≈ 0.8) with the themselves strongly correlated pattern related indices VLF/HF, LF/HF and SD/RM.
In the P ATTERN category, the different aspects like skewness, sympatho-vagal balance (VLF/LF, LF/HF, SD/RM, LF/HF), and time irreversibility (P*, G*, E*) were not correlated. However, the three Asym indices were mutually correlated at similar time scales (P1-G1; r= 0.68, E1-G1: r = 0.88, P4-G4: r = 0.50, E4-G4: r = 0.82). Inside the sympatho-vagal balance parameters VLF/LF seems to stand alone in comparison the other three.

Figure 1: correlation matrix

<INSERT FIGURE 1>

In order to check for the exclusivity of the selected representatives of categories we compared the standard errors of the best age-predicting representatives with the best predicting couple. The improvements of SE were less than 0.1 WGA and, hence, irrelevant compared to the clinical precision of the age determination with a confidence interval of ± 1 WGA ((Geirsson, 1991), table 3). SE of COMP was decreased by 0.5 towards 3.7 in the combination of MSE4 and α2. Therefore, we used only MSE4 for that category as candidates for the minimal set.

Table 3: standard errors SE(standard deviation SD of SE) / coefficient of determination R^2 in WGA of minimal set members of classifiers extracted below, best single and best couple of age predicting indices, * indicates significant difference between best single age predictor and classification representative, # indicates significant difference between best single and couple age predictor.

<INSERT TABLE 3>

Representatives of categories with respect to identification of different deviations

It was our intention to find an appropriate minimal set of category representatives that allows the identification of all investigated deviations. Table 4 shows, that the best deviation class discriminating indices were significantly different compared to the age prediction representatives. However, the improvements by using couples of indices were mainly below an AUC difference of 0.05 and could primarily reached in investigations at younger ages, like in DHA (24 WGA) and IUGR (both in Jena and Bochum before 32). In studies after or around 32 WGA, nearly no significant improvements could be reached. Accepting this minor loss of precision at the younger ages, a minimal screening set could be established by just one representative per category. For this objective, the same representatives across all kinds of deviations are required. The AUC values of the respective set [ACst1, STV, LZC] we reduced by a maximum of 0.06 in comparison to the best results. Therefore, we did not include any of them in the minimal set.

Table 4: Classification results: Area under curve (AUC) and 95% confidence interval (CI) per category: age prediction representative vs. best classifiers vs. best predicting couple, minimal set of overarching classifiers. * indicates significant difference between best single classification feature and the best age predicting feature, # indicates significant difference between best single and couple of classification feature. OGTT: significances of differences and interactions.

<INSERT TABLE 4>

Minimal set for overarching screening of maturation age and deviations

While maturation age was related to both [Amp, pNN5, MSE4, skew], all investigated deviations could be screened for by at least one of [ACst1, STV, LZC].

Since ACstep1 and pNN5 predict maturation age almost similarly (table 3), but ACstep1 better discriminated most of the deviation aspects (table 4), we propose ACstep1 as overarching representative of sAMP.
STV and Amp predict maturation age almost similarly (table3), but STV better discriminated most of the deviation aspects (table 4). Furthermore, STV is common in established CTG analysis and allows the transfer of results obtained by MCG to the established CTG recordings and standards. Therefore, we propose STV as overarching representative of lAMP.
Because LZC did not significantly predict age we propose to keep MSE4 as (lCOMP) age predicting feature while LZC as (sCOMP) classification feature representative.

The resulting overarching minimal set includes
[STV, ACst1, MSE4, skew] for the maturation age prediction and
[STV, ACst1, LZC] for the discrimination of other deviations.

Assessment of maturation age across 2d and 3d trimester by minimal set [**STV, ACst1, MSE4, skew*****]***

The results shown in Figure 2, indicate that the most profound maturation related changes appeared up to the investigation range of (26-32) WGA, namely increasing values (positive regression coefficients) of lAMP, sAMP, lCOMP indices and the pattern index skewness. In contrast, after a transition period at the range of (29-35) WGA, the indices of both AMP categories become again strong predictors, but the COMP and pattern index lose their relevance.
The Bochum data qualitatively confirm these results. Lower significances can be explained by the higher variability of the state-independent and shorter (5 min) recordings.

Figure 2: Normal group of Jena data base, linear regression models describing HRV categories by fetal WGA, standardized regression coefficient Beta, 95 % CI of GA intervals: range 23 (20-26), range 26 (23-29), range 29 (26-32), range 32 (29-35), range 35 (32-38).
<INSERT FIGURE 2>

Assessment of deviations by influencing factors by minimal set [*STV, ACst1, LZC*]

In Table 5, significant changes due to influencing factors are shown. The corresponding statistical metrics are given in Table 4. Please note that the data sets were obtained by availability. Consequently, we were not able to investigate the different influencing factors for all developmental periods. Nevertheless, we found systematic examples of significant discriminatory power for each HRV category.

Table 5: HRV categories representatives significantly associated with the influencing factors: ↑/↓: significant increase/decrease compared to normal group due to corresponding factor, - : no association, empty fields: no data. The corresponding quantitative statistics in detail are given in the Table 4. §: reduced at third measurement time point.

<INSERT TABLE 5>

Compared to the results in the above section on age dependency, we did not find a general change from predominantly discriminating HRV complexity and amplitude before 32 WGA to a solely predominantly discriminating HRV amplitude after 32 WGA. However, it is important to note that there seems to be a dominance of amplitude changes due to maternal life-style factors (physical exercise, smoking) in contrast to both amplitude and complexity changes in connection with fetal growth (IUGR) and nutrient supplementation (DHA).

Discussion

The myriad and diverse published HRV indices prevent their standardized application in routine clinical evaluation of fetal maturation, its pathophysiological disturbances as well as of behavioral influences. The present work aimed at the extraction of a clear set of a few HRV representatives capable for an overarching monitoring/screening of as many of those aspects as possible. All familiar HRV indices were organized in categories according to physiological and signal-theoretical aspects and the members of each category were checked for redundancies and synergism with respect to physiological maturation age and deviations from the normal values.
Across all aspects of deviations we suggest overlapping sets of:
[lAMP(STV), sAMP(ACst1), lCOMP(MSE4), PATTERN(skewness)] for maturation age prediction and
[lAMP(STV), sAMP(ACst1), sCOMP(LZC)] for the discrimination.
It is remarkable that only in a few cases multivariate intra-category models weakly improved the predictive value of particular deviations. This was mainly the case when considering fetuses below 32 WGA and could be an expression of more complex system behavior and development during this period. This could be of interest in subsequent diagnosis in more detail after identification of suspect cases using the overarching screening methodology presented here. However, with respect to the screening we could not find overarching multivariate intra-category models. The merging of categories was no option since they consider different aspects of physiological system behavior.
In the present work we found changing relevance of HRV indices with respect to the GA with a transition range around 32 WGA (29-35 WGA). In terms of nonlinear dynamics, this phenomenon can be interpreted as a typical phase transition period where the signal becomes less clearly assignable at the edge between the previous and the subsequent maturation period. Also, from a physiological point of view, the change from the predominant formation and growth of vagal and sympathetic complex structures before 32 WGA towards the increase of behavioral patterns of the mainly developed complex organism after 32 WGA indicates a phase transition. A corresponding transition range has been reported in the development of the HRV power spectra and complexity indices (Van Leeuwen *et al.*, 2003; Hoyer *et al.*, 2017). In a previous study using a former recruitment state of the Jena data base of 312 recordings from 60 women using multivariate linear mixed models over 4 maturation segments (<27, 27-31, 31+1-35, >35+1 WGA) we found qualitatively similar relationships of corresponding HRV indices (Schneider *et al.*, 2018). The previously described components of fABAS found by linear regression, namely Amp, pNN5, MSE3, skew (Hoyer *et al.*, 2013b) perfectly fit with the category representatives elaborated in this study: lAMP: Amp≈STV, sAMP: pNN5≈ACst1, lCOMP: MSE4, PATTERN: skew. The similar analysis applied to the independent Bochum 5 min normal group recordings, confirmed the main characteristics of the HRV categories.

The examples of deviations investigated are the result of our international search for appropriate data sets with heart rate patterns of MCG quality. The data sets were independent between the study centers but constitute only an incomplete survey.
Due to the fundamental limitations of ECG recordings, only a few acceptable recordings were available and did not allow a statistical analysis. They did however indicate similar qualitative results. CTG recordings were not considered due to their lower time resolution and will be addressed in a subsequent study. The categories of long-term fluctuation amplitude and complexity can be considered as transferable to CTG data. In contrast, the short-term categories are not yet

transferable since they are based on individual heart beat intervals that can so far not reliably be identified by the established CTG technology.

In all deviation data sets, we found that the HRV categories investigated here are correlated with the established HRV indices found in the original analyses of the data sets or similar analyses using other data sets.

- In fetuses of women doing aerobic exercise, all classical time and frequency domain fetal HRV indices were increased compared to fetal HRV of passive women at 36 WGA when fetuses were in the active sleep state (May *et al.*, 2010). Consistent with the initial report, the current re-analysis of this data set showed larger values of the category lAMP.
- Acute maternal cigarette smoking reduced fetal HRV (Graca *et al.*, 1991; Peterfi *et al.*, 2017). These results are in line with the reduced lAMP category in the present work.
- Maternal DHA supplementation increased SDNN, RMSSD, VLF, LF, (HF trend) (Gustafson *et al.*, 2013). It also increased fABAS, the maturation age score that includes indices of sAMP (pNN5), lAMP (ActAmp), and lCOMP (MSE3) (Hoyer *et al.*, 2018). Consistent with previously reported findings, the present work further expands our understanding, showing increased values of both fluctuation amplitude and complexity.
- In IUGR fetuses reduced HRV indices were shown that correspond to lAMP using CTG recordings (Nijhuis *et al.*, 2000). Decreased HRV complexity in IUGR fetuses has been described in CTG recordings (Lyapunov Exponent (LE) (Kikuchi *et al.*, 2008), Lempel Ziv complexity (LZC) and multiscale entropy (MSE) of Approximate Entropy (ApEn) and Sample Entropy (SampEn) (Ferrario *et al.*, 2009)) across the age range from 27 to 34 WGA. It should be noted that these indices of complexity, obtained from the CTG based heart rate signal, correspond to long-term behavior. Consistently, in the present work representatives of the lAMP and lCOMP categories were reduced. It should be noted, that furthermore the sAMP representative was decreased. Accordingly, fABAS that contains lAMP, sAMP and lCOMP was previously found reduced in IUGR fetuses (Hoyer *et al.*, 2013b).
- Reduced heart rate variability was observed in fetuses of mothers with GDM 120 minutes after the OGTT (Fehlert *et al.*, 2017). This is in accordance with the current finding of lAMP.

The choice of active sections (2F) is consistent with CTG recommendations and can be recommended in comparison to the more disperse and less defined non-state-classified recordings as well as the relatively rare quiet sleep (1F) sections. Our analyses of 1F and state-independent data (apart 5min Bochum data) of all influencing factors were not outlined in the manuscript.

The elaborated minimal set of HRV indices was intended to be appropriate for routine overarching screening/monitoring. A resulting “polyscore” (e.g.(Steger *et al.*, 2019)) could identify suspect cases if one or more of the proposed category representatives is out of the normal range. This does not exclude the possibility that in identified suspect cases subsequent multivariate models using further indices such as power spectral ratios, α, or time irreversibility indices, including those from different categories, may consider higher discriminatory complex interrelationships. However, those particular models are not helpful for the here proposed universal overarching monitoring.

Conclusion

The precise identification of fetal developmental disturbances by means of HRV indices requires the appropriate consideration of standardized representatives of HRV key characteristics under consideration of two factors, namely the maturation age and the disturbance of interest. For that purpose, the HRV categories short- and long-term fluctuation amplitude and short- and long-term fluctuation complexity integrate the most relevant HRV indices. The standardized use of representatives of these categories in a corresponding multivariate approach could help to establish a generally valid standardized diagnostic tool. We propose a multivariate screening discriminator that identifies changes in representatives of the categories lAMP, sAMP, lCOMP, sCOMP, and PATTERN. This should be confirmed and possibly refined in subsequent analyses of independent data sets. Any contributing study center is welcome to collaborate.

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List of Abbreviations and HRV parameters:

For details and references of HRV indices see Table 2.

ANS – autonomic nervous system
AUC – area under curve
CI – confidence interval
CTG – cardiotocography
DHA – docosahexaenoic acid
ECG – electrocardiogram
fABAS – fetal autonomic brain age score
GDM – gestational diabetis melitus
HR – heart rate
HRV – heart rate variability
IUGR – intrauterine growth restriction
MCG – magnetocardiography
(W)GA – (weeks) gestational age

HRV Parameters

Amp – amplitude range: 20-95 inter quantile distance of detrended NN interval series
DCslx, DCstx, ACslx, ACstx – deceleration capacity and acceleration capacity, slope and step value at coarse graining level x
DFA – detrended fluctuation analysis: scaling indices α1, α2EIx – Ehler's index of temporal asymmetry at coarse graining level x
GIx – Guzik's index of temporal asymmetry at coarse graining level x
HF – fetal high frequency band (0.4-1.7 Hz)
LF – fetal low frequency band (0.08-0.2 Hz)
LTV – long-term variability
LZC – Lempel Ziv complexity of binary transformed NN intervals
mHR – mean fetal heart rate
MSEx – generalized multiscale entropy at coarse graining level x
PIx – Porta's index of temporal asymmetry at coarse graining level x
pNN5 – percentage of differences between adjacent NN intervals exceeding 5 ms
PRSA – phase rectified signal averaging
PxV – patterns with x variation of binary transformed NN intervals
RM – RMSSD – root mean square of successive NN interval differences
SD – SDNN – standard deviation of NN intervals
skew – skewness
STV – short-term variability
TP – fetal total frequency power
VLF – fetal very low frequency band (0.02-0.08 Hz)

Figures without legend

Legends are included in the text above

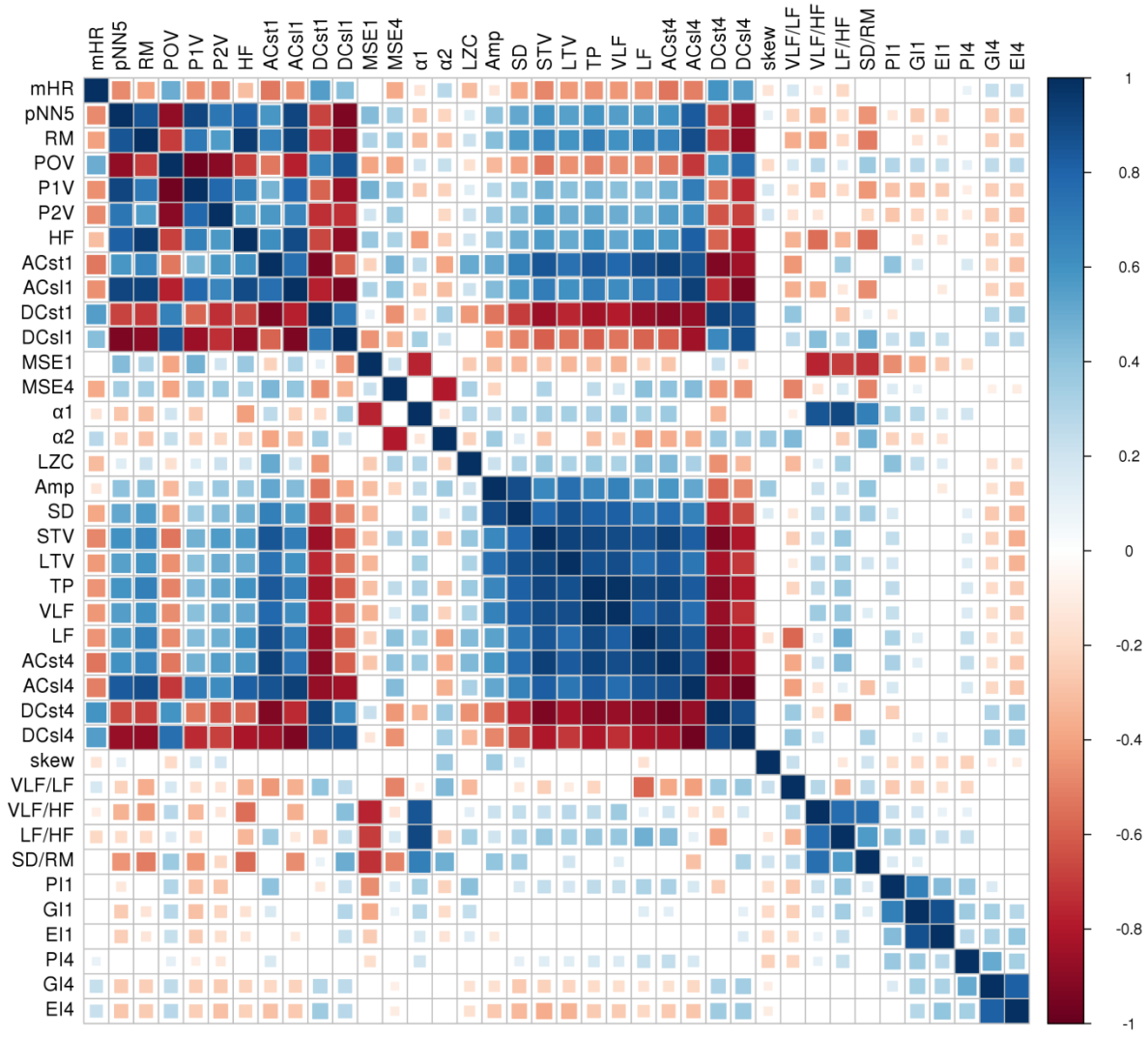

Figure 1

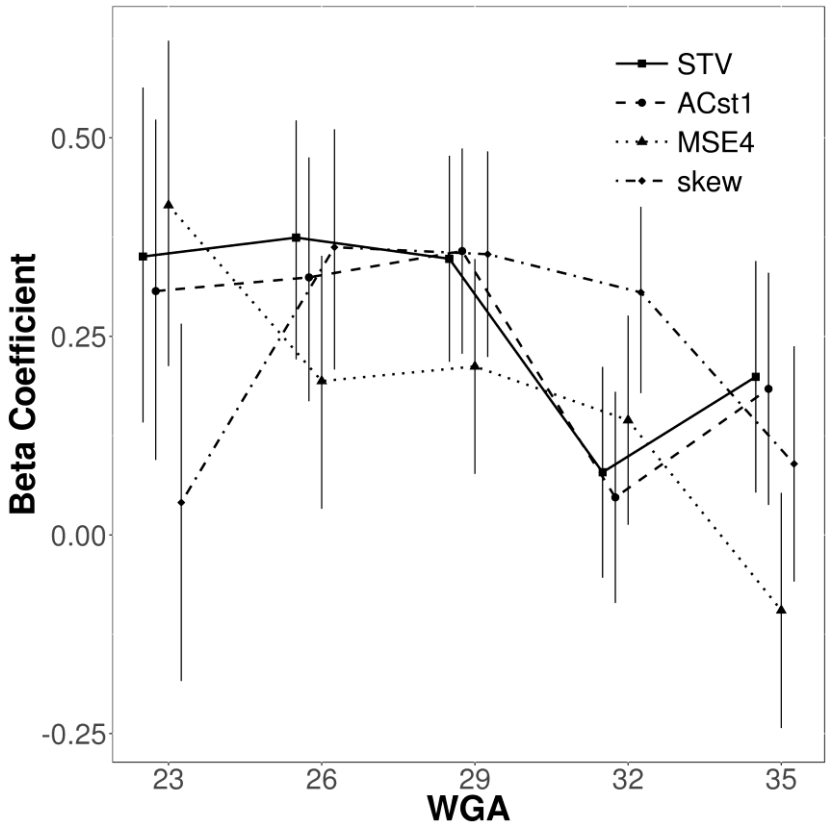
STV
ACst1
MSE4
skew
0.50
0.25
0.00
-0.25
Beta Coefficient
23
26
29
32
35
WGA

Figure 2

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Table 1

Signal	Center Location	Group	Number available records	Number analysed records 2F	Recording duration	WGA
MKG	Jena	Normal	567	484	30 min	18-40
		IUGR	48	34	30 min	26-38
	Bochum	Normal	305	296	5 min	15-42
		IUGR	78	49	5 min	19-40
	Tübingen	Normal	108	89	15 min	28-36
		GDM	39	31	15 min	28-36
	Kansas	Normal	19	12	18 min	36
		Exercise	21	15	18 min	36
	Kansas	Normal	68	60	2x18 min	24/32/36
		DHA	66	63	2x18 min	24/32/36
	Kansas	Pre-Smoking	24	20	18 min	24/28/30/32/34/36/38
		Post-Smoking	24	17	18 min	24/28/30/32/34/36/38
ECG	Kharkiv	Normal	74	45	10-30 min	20-36
		IUGR	13	5	10-30 min	20-36

Table 2

AMP: Magnitude (amplitude): indices of the range of fluctuations dependent on different time resolutions or frequency bands		
short (sAMP)	RM: RMSSD- Root Mean Square of Successive beat Differences	(TaskForce, 1996)
	pNN5: percentage of successive NN intervals greater than 5 ms	(TaskForce, 1996)
	HF (high frequency band power)	(David *et al.*, 2007)
	P0V, P1V, P2V: percentage of no, one, or two respective changes in a binary series of successive NN intervals	(Cysarz *et al.*, 2015; Cysarz *et al.*, 2013)
	DCsl1, DCst1, ACsl1, ACst1: Slope and step values of heart rate Deceleration and Acceleration Capacity across 1 NN interval change (short) after PRSA (Phase Rectified Signal Averaging)	(Bauer *et al.*, 2006; Lobmaier *et al.*, 2012)
long (lAMP)	SD: SDNN -standard deviation of NN intervals	(TaskForce, 1996)
	TP: total power, VLF: very low frequency p., LF: low frequency p.	(David *et al.*, 2007)
	Amp: 20-95 inter-quantile range of NN intervals	
	STV: mean difference between consecutive NN interval epochs of 3.75s, w/o DC, artifacts <50%, the parameter is referred to as STV - 'short term variation' in the literature owing to the temporal resolution constrains of cardiotocography	(Pardey *et al.*, 2002)
	LTV: "long-term variation", mean fluctuation range of NN epochs in 1 min sections w/o DC, artifacts < 50%, part of the dataset referred to as the Dawes-Redman-criteria	(Pardey *et al.*, 2002)
	DCsl4, DCst4, ACsl4, ACst4, slope and step values across the change at a coarse graining level of 4 NN intervals (long)	see above
COMP: Complexity: indices of irregularity, invers to predictability. Complexity is a characteristic that is per definition independent of the amplitude of a signal.		
LZC: Lempel Ziv Complexity (short)		(Lempel and Ziv, 1976)
MSE: Multi-Scale Entropy, calculated from generalized mutual information from original NN series, scale 1 (short), scale 4 (long), equivalent to approximate entropy and sample entropy		(Richman and Moorman, 2000) (Hoyer *et al.*, 2013a)
α1: fractal scaling index: across 4-11 NN (short), α2: - across > 11 NN intervals (long) from DFA (Detrended Fluctuation Analysis)		(Peng *et al.*, 1995)
PATTERN: indices derived from nonlinear distributions or ratios. They are in the most cases per definition independent of the amplitude or complexity of the original signal.		
skew: skewness (third statistical moment, asymmetry of the probability distribution of a random variable such as NN interval series)		
VLF/LF, VLF/HF, LF/HF (ratios of frequency band power) SD/RM: SDNN/RMSSD (corresponding ratio of time domain indices)		(David *et al.*, 2007) (Schneider *et al.*, 2008)
Multiscale temporal asymmetry (short scale 1, long scale 4) PI1, PI4 (Porta's index, percentage of negative values in the [NN(i), NN(i+1)] map normalized to the total number of values) GI1, GI4 (Guzik's index, cumulative distance of [NN(i), NN(i+1)] values above the main diagonal normalized to the overall cumulative distance) EI1, EI4 (Ehler's index, skewness of [NN(i), NN(i+1)] distribution)		(Porta *et al.*, 2008)

Table 3

category	classification feature representative SE(SD)/R^2	best age predicting feature SE(SD)/R^2	best age predicting couple SE(SD)/R^2
Jena (18-40 WGA)			
sAMP	3.89(0.13)/0.26 (ACst1)	3.67(0.15)/0.35 (pNN5)*	3.57(0.16)/0.38 (pNN5+RM)#
lAMP	3.83(0.14)/0.29 (STV)	3.55(0.14)/0.39 (Amp)*	3.46(0.16)/0.42 (AMP+DCsl4)#
COMP	4.49(0.11)/0.02 (LZC)	4.21(0.15)/0.13 (MSE4)*	3.70(0.18)/0.34 (MSE4+α2)#
Patterns		3.82(0.15)/0.29 (skew)	3.77(0.16)/0.31 (skew+VLF/HF)#
Bochum (15-42 WGA)			
sAMP	5.38(0.28)/0.34 (ACst1)	4.98(0.33)/0.43 (P0V)*	4.93(0.31)/0.44 (pNN5+DCsl1)#
lAMP	5.74(0.23)/0.24 (STV)	5.15(0.31)/0.39 (DCsl4)*	5.10(0.26)/0.41 (STV+DCst4)
COMP	6.24(0.20)/0.11 (LZC)	5.78(0.31)/0.24 (MSE4)*	5.54(0.32)/0.30 (MSE4+α2)#
Patterns		5.74(0.28)/0.24 (skew)	5.33(0.29)/0.35 (skew+VLF/HF)#

Table 4

category	age predicting feature representative	classification feature representative	best classification feature	best classification couple
Physical exercise (36 WGA)				
sAMP	.70(.65-.75)(pNN5)	.75(.70-.80)(ACst1)	.75(.70-.80)(ACst1) *	.70(.66-.75)(HF+ACst1) #
lAMP	.78(.74-.83)(Amp)	.77(.73-.81)(STV)	.83(.83-.87)(VLF)	.85(.81-.89)(VLF+DCst4)
COMP	.35(.31-.38)(MSE4)	.68(.62-.75)(LZC)	.71(.66-.75)(MSE1) *	.66(.61-.71)(MSE4+α1)
PATTERN	.33(.27-.39)(skew)		.62(.55-.70)(PI4) *	.70(.65-.75)(GI4+EI4) #
DHA supplement (24 WGA)				
sAMP	.38(.33-.42)(pNN5)	.45(.40-.50)(ACst1)	.45(.40-.50)(ACst1) *	.57(.52-.63)(pnn5+P0V) #
lAMP	.39(.35-.43)(Amp)	.41(.38-.45)(STV)	.52(.47-.57)(LF) *	.59(.53-.65)(STV+LF) #
COMP	.56(.49-.63)(MSE4)	.37(.34-.41)(LZC)	.56(.49-.63)(MSE4)	.60(.55-.65)(MSE4+α2)
PATTERN	.59(.53-64)(skew)		.67(.64-.71)(EI4) *	.70(.66-.73)(EI1+EI4)
DHA supplement (32 WGA)				
sAMP	.73(.69-.78)(pNN5)	.74(.69-.79)(ACst1)	.80(.76-.83)(P2V) *	.78(.74-.82)(RM+P2V)
lAMP	.55(.51-.60)(Amp)	.71(.67-.76)(STV)	.75(.71-.80)(DCsl4)*	.74(.70-.79)(DCst4+DCsl4)
COMP	.67(.60-.73)(MSE4)	.77(.73-.82)(LZC)	.77(.73-.82)(LZC) *	.76(.76-.81)(LZC+α1)
PATTERN	.46(.42-51)(skew)		.57(.51-.63)(PI4) *	.60(.56-.64)(PI4+EI4)
DHA supplement (36 WGA)				
sAMP	.68(.64-.73)(pNN5)	.71(.67-.76)(ACst1)	.74(.69-.78)(P2V) *	.74(.70-.78)(P1V+P0V)
lAMP	.76(.73-.80)(Amp)	.72(.67-.76)(STV)	.82(.79-.85)(SD) *	.80(.77-.84)(Amp+SD)
COMP	.43(.38-.48)(MSE4)	.69(.64-.73)(LZC)	.69(.64-.73)(LZC) *	.70(.66-.74)(MSE4+LZC)
PATTERN	.44(.40-.48)(skew)		.60(.55-.65)(PI4) *	.62(.58-.67)(SD/RM+PI4)
IUGR (Jena, < 32 WGA)				
sAMP	.42(.38-.47)(pNN5)	.71(.67-.74)(ACst1)	.71(.67-.74)(ACst1) *	.78(.76-.80)(P2V+DCsl1) #
lAMP	.51(.45-.57)(Amp)	.72(.68-.76)(STV)	.73(.70-.77)(ACst4) *	.77(.72-.77)(DCst4+DCsl4)#
COMP	.65(.61-.68)(MSE4)	.75(.72-.78)(LZC)	.75(.72-.78)(LZC) *	.75(.73-.79)(LZC+α2)
PATTERN	.44(.41-.48)(skew)		.69(.66-.73)(LF/HF)*	.69(.58-.62)(VLF/HF+SD/RM)
IUGR (Jena, >= 32 WGA)				
sAMP	.66(.62-.69)(pNN5)	.69(.67-.72)(ACst1)	.69(.67-.72)(ACst1)*	.70(.67-.72)(RM+HF)
lAMP	.67(.65-.70)(Amp)	.76(.73-.79)(STV)	.76(.73-.79)(STV) *	.76(.73-.78)(STV+TP)
COMP	.58(.53-.62)(MSE4)	.59(.54-.64)(LZC)	.59(.54-.64)(LZC)	.61(.57-.64)(MSE1+MSE4)
PATTERN	.58(.54-.62)(skew)		.63(.60-.66)(PI1) *	.68(.65-.72)(PI1+EI1) #
Bochum (Jena, < 32 WGA)				
sAMP	.51(.47-.56)(pNN5)	.60(.56-.64)(ACst1)	.60(.56-.64)(ACst1) *	.73(.71-.75)(P2V+ACsl1)#
lAMP	.54(.51-.58)(Amp)	.60(.56-.64)(STV)	.66(.63-.70)(VLF) *	.77(.74-.80)(Amp+TP) #
COMP	.53(.47-.59)(MSE4)	.47(.44-.50)(LZC)	.67(.62-.71)(α2) *	.64(.60-.68)(MSE1+α2) #
PATTERN	.64(.61-.66)(skew)		.72(.69-.75)(EI1) *	.75(.72-.77)(SD/RM+EI1)
Bochum (Jena, >= 32 WGA)				
sAMP	.64(.60-.67)(pNN5)	.63(.61-.66)(ACst1)	.67(.64-.71)(P1V) *	.65(.62-.69)(HF+ACsl1)
lAMP	.50(.46-.54)(Amp)	.55(.51-.58)(STV)	.64(.60-.67)(DCsl4) *	.67(.64-.69)(VLF+TP)
COMP	.59(.55-.62)(MSE4)	.49(.45-.52)(LZC)	.62(.59-.65)(MSE1)	.61(.57-.64)(α1+α2)
PATTERN	.57(.54-.60)(skew)		.67(.65-.70)(VLF/HF) *	.68(.66-.71)(skew+VLF/HF)
Pre-Post Smoke				
sAMP	.26 (pNN5)	.16 (ACst1)	.08 (RM)	-
lAMP	.23 (Amp)	.05 (STV)	.05 (STV)	-
COMP	.67 (MSE4)	.48 (LZC)	.48 (LZC)	-
PATTERN	.21 (skew)		.21 (skew)	-
OGTT				
sAMP	.013/.407 (pNN5)	.010/.348 (ACst1)	.002/.193 (HF)	-
lAMP	n.s. (Amp)	.002/.123 (STV)	.060/.056 (LF)	-
COMP	.026/.238 (MSE4)	n.s. (LZC)	.001/.734 (MSE1) .016/.067 (MSE5)	-
PATTERN	n.s. (skew)		.001/.276 (VLF/HF)	-

Table 5

	20-32 WGA				range 32 (29-35) WGA				32-38 WGA			
HRV category vs. influencing factor	HR	sAMP	lAMP	COMP	HR	sAMP	lAMP	COMP	HR	sAMP	lAMP	COMP
Physical exercise 36 WGA									-	↑	↑	-
DHA supplement. 32WGA 36WGA					-	↑	↑	↑	-	↑	↑	↑
IUGR, Jena	↑	↓	↓	↓	(↑) 0.07	↓	↓	↓	↑	↓	↓	-
IUGR, Bochum	↑	-	(↓) 0.08	-	↑	↓	-	-	-	-	-	-
OGTT 28-36 WGA					-	↓§	↓§	-				
Pre-post smoking 24 – 38 WGA					-	-	↓	-				