Archives of Gynecology and Obstetrics
https://doi.org/10.1007/s00404-019-05390-8

MATERNAL-FETAL MEDICINE

Fetal heart rate variability responsiveness to maternal stress, non-invasively detected from maternal transabdominal ECG

Silvia M. Lobmaier[1] · A. Müller[2] · C. Zelgert[1] · C. Shen[3] · P. C. Su[3] · G. Schmidt[2] · B. Haller[4] · G. Berg[5] · B. Fabre[6] · J. Weyrich[1] · H. T. Wu[7,8,9] · M. G. Frasch[10] · M. C. Antonelli[1,11]

Received: 14 July 2019 / Accepted: 14 November 2019

Abstract

Purpose Prenatal stress (PS) during pregnancy affects in utero- and postnatal child brain-development. Key systems affected are the hypothalamic–pituitary–adrenal axis and the autonomic nervous system (ANS). Maternal- and fetal ANS activity can be gauged non-invasively from transabdominal electrocardiogram (taECG). We propose a novel approach to assess couplings between maternal (mHR) and fetal heart rate (fHR) as a new biomarker for PS based on bivariate phase-rectified signal averaging (BPRSA). We hypothesized that PS exerts lasting impact on fHR.

Methods Prospective case–control study matched for maternal age, parity, and gestational age during the third trimester using the Cohen Perceived Stress Scale (PSS-10) questionnaire with PSS-10 over or equal 19 classified as stress group (SG). Women with PSS-10 < 19 served as control group (CG). Fetal electrocardiograms were recorded by a taECG. Coupling between mHR and fHR was analyzed by BPRSA resulting in fetal stress index (FSI). Maternal hair cortisol, a memory of chronic stress exposure for 2–3 months, was measured at birth.

Results 538/1500 pregnant women returned the questionnaire, 55/538 (10.2%) mother–child pairs formed SG and were matched with 55/449 (12.2%) consecutive patients as CG. Maternal hair cortisol was 86.6 (48.0–169.2) versus 53.0 (34.4–105.9) pg/mg ($p = 0.029$). At 36 + 5 weeks, FSI was significantly higher in fetuses of stressed mothers when compared to controls [0.43 (0.18–0.85) versus 0.00 (− 0.49–0.18), $p < 0.001$].

Conclusion Prenatal maternal stress affects the coupling between maternal and fetal heart rate detectable non-invasively a month prior to birth. Lasting effects on neurodevelopment of affected offspring should be studied.

Trial registration Clinical trial registration: NCT03389178.

Keywords ANS · Bivariate phase-rectified signal averaging · BPRSA · Fetal autonomic nervous system · Fetal heart rate · Fetal stress index · FSI · Prenatal stress · PS

Introduction

Prenatal exposure to maternal psychosocial stress and anxiety confers lifelong risk for behavioral alterations that last beyond childhood [1, 2]. Every fifth-to-fourth pregnant woman experiences such prenatal stress (PS) [3] which can impact on early behavioral, cognitive development, and temperament in human infants, and increases child morbidity and neurological dysfunction [2, 4]. In a recent review, Van den Bergh et al. [2], conclude that numerous epidemiological and case–control studies show neurodevelopmental disorders in offspring exposed to maternal stress during pregnancy. Pregnant women that were exposed to psychosocial stress during the third trimester of pregnancy have infants (5–18 months of age) that show: less infant affective reactivity at 5 months [5], higher infant temperamental reactivity at 6 months [6], positive association in infants with high respiratory sinus arrhythmia at 8–10 months [7], higher infant negative affectivity at 24 months [8], and higher reaction intensity in children at 24–30 months [9], implying that the main outcome is at the cognitive- and temperamental levels.

Considering the difficulty to differentiate the concept of stress (including the psychological assessment of perceived

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00404-019-05390-8) contains supplementary material, which is available to authorized users.

✉ Silvia M. Lobmaier
silvia.lobmaier@tum.de

Extended author information available on the last page of the article

Published online: 28 November 2019

stress) from anxiety, we will employ the term 'psychosocial stress' to refer to maternal general- and pregnancy-specific stress and anxiety throughout this study [10, 11]. The mechanisms of the psychosocial stress response can be divided into an acute response, which involves the rapid activation of the autonomic nervous system (ANS), and a delayed response mediated by the hypothalamic–pituitary–adrenal (HPA) axis. The neurally mediated ANS responses enable precise adjustments of target organs within seconds, while the HPA's slow response results in peak levels of cortisol within 10–30 min after acute stressor [12]. Most importantly, these maternal stress responses shape the development of the infant's stress response system, a phenomenon referred to as "fetal programming". Although this field still faces the challenge of thoroughly understanding the underlying pathophysiological mechanisms [13] the HPA/ANS responses are still considered to be the main mechanism by which prenatal exposures influence human-postnatal development [14]. The quest for finding a prenatal measure that might have a preventive clinical significance [15] has led us to hypothesize that the coordinated roles of the ANS and the HPA in the integrated stress response can be monitored non-invasively using electrocardiogram (ECG) and ECG-derived maternal- and fetal heart-rate (mHR, fHR). The relationship between mHR and fHR might provide important information about the functional status of fetal ANS [16]. In a prospective cohort of late-gestation women, we tested whether a new biomarker measuring the coupling of mHR and fHR can predict the preceding chronic exposure of mothers to stress. Herein, we propose a novel analysis method of coupling between mHR and fHR based on a signal-processing algorithm, first applied in adult cardiology, termed bivariate phase-rectified signal averaging (BPRSA) [17, 18] and applied to trans-abdominally acquired fetal ECG (fECG). This method overcomes the limitation of non-stationary signal and background noise typical for fHR signal.

Such physiological biomarkers could serve as foundation for prediction of the child's neurodevelopmental outcomes and aid in devising early developmental intervention strategies for children at risk of altered neurodevelopmental trajectories due to prenatal stress exposure. The aim of the study was to: (1) evaluate the coupling between mHR and fHR by BPRSA analysis; and (2) compare BPRSA results of fetal response to mHR changes in healthy controls and stressed fetuses.

Materials and methods

Study design and study population

We performed a prospective matched control study in stressed mothers with controls matched for 1:1 for parity, maternal age, and gestational age at study entry. Subjects were recruited for 22 months (July 2016 until May 2018) from a cohort of pregnant women followed in the Department of Obstetrics and Gynecology at "Klinikum rechts der Isar" of the Technical University of Munich (TUM), a tertiary center of Perinatology located in Munich, Germany, serving ~ 2000 mothers/newborns per year.

Experimental design

TUM obstetricians identified prospective subjects according to the following inclusion criteria: women with singleton pregnancies between 18 and 45 years of age in their third trimester (at least 28 weeks gestation). Exclusion criteria were (a) serious placental alterations defined as fetal growth restriction according to Gordijn et al. [19]; (b) fetal malformations; (c) maternal severe illness during pregnancy (Table 1 from [20]); (d) maternal drug or alcohol abuse.

The participants entered Phase I–III of the study:

Phase I: Screening

We administered Cohen Perceived Stress Scale questionnaire (PSS-10) [21] to all pregnant women visiting the outpatient ward of the Department of Obstetrics and Gynecology at "Klinikum rechts der Isar" of the Technical University of Munich, attached to a short information brochure about the study. 1500 questionnaires were distributed during the study period. PSS-10 categorized them as SG for PSS-10 score ≥ 19 [3]. Inclusion- and exclusion criteria were applied after returning the questionnaires. For every subject categorized as stressed, the next screened participant matching for gestational age at recording with a PSS-10 score < 19 was entered into Phase II as control. Due to limited staff resources, not all controls could be included into phase II of the study, so we choose the 1:1 matching criteria as described above.

Measurement of maternal stress during pregnancy: Maternal psychosocial stress was measured using the Cohen Perceived Stress Scale (PSS 10). This questionnaire measures the degree to which situations in one's life are appraised as stressful and is a widely used psychological instrument to measure nonspecific perceived stress [21]. The PSS-10 predicts objective biological markers of stress and increased risk for disease among persons with higher perceived stress levels. Increased maternal prenatal stress, measured by PSS-10, was associated with temperamental variation of young infants and may represent a risk factor for psychopathology later in life [22]. The PSS-10 has been validated in German speaking populations and is a quick tool for screening stress among prospective subjects [23–25].

Table 1 Study outcome parameter

Characteristics	Control $n=55$	Prenatal stress $n=55$	p
Baseline			
Gestational age at screening (weeks)	34.0 (33.4–35.0)	34.0 (32.7–35.1)	0.626
Gestational age at inclusion (weeks)	36.7 (35.0–37.4)	36.4 (35.4–37.4)	0.844
Maternal age (years)	35.2 (3.5)	33.8 (5.4)	0.108
BMI pregestational (kg/m^2)	21.5 (20.2–23.5)	24.2 (20.9–30.8)	0.001*
BMI at inclusion (kg/m^2)	26.1 (24.5–28.7)	29.8 (26.0–36.7)	< 0.001*
European/Caucasian	50 (90.9)	51 (92.7)	0.728
Married	41 (74.5)	39 (70.9)	0.669
University degree	45 (81.8)	29 (52.7)	0.001*
Household income > 5000€/month	35 (63.6)	19 (34.5)	0.002*
Smoking	1 (1.8)	7 (12.7)	0.028*
Multiparity	22 (40.0)	30 (54.5)	0.127
Planned pregnancy	50 (91.0)	36 (65.5)	0.001*
IVF/ICSI	6 (10.9)	2 (3.6)	0.142
Gestational diabetes	1 (1.8)	9 (16.4)	0.008*
Autoimmune disease	2 (3.6)	10 (18.2)	0.014*
Working status at screening	2 (3.6)	4 (7.2)	0.388
Score PSS-10	9.0 (6.0–12.0)	22.0 (21.0–24.0)	< 0.001*
Cortisol in maternal hair (pg/mg)[a]	53.0 (34.4–105.9)	86.6 (48.0–169.2)	0.029*
Maternal heart rate (bpm)	87.0 (10.6)	88.7 (9.3)	0.382
Maternal respiratory rate	27.9 (3.6)	28.4 (3.6)	0.437
Fetal heart rate (bpm)	140 (136–146	140 (136–147)	0.995
FSI (ms)	0.00 (-0.49–0.18)	0.43 (0.18–0.85)	< 0.001*
Perinatal outcome			
Gestational age at delivery (weeks)	40.0 (39.0–40.7)	39.4 (38.6–40.6)	0.058
Cesarean delivery (CD)	10 (18.2)	23 (41.8)	0.007*
"Planned" CD	3 (5.4)	14 (25.4)	< 0.001*
CD after onset of labor	7 (12.8)	9 (16.4)	0.470
Gender female	24 (43.6)	20 (36.45)	0.436
Birthweight (g)	3560 (412)	3552 (470)	0.922
Birthweight percentile	52.1 (25.0)	57.6 (25.7)	0.270
Length (cm)	53.0 (51.0–55.0)	53.0 (52.0–55.0)	0.591
Head circumference (cm)	35.0 (34.0–36.0)	35.0 (34.0–36.0)	0.437
Apgar min 5	10.0 (9.0–10.0)	10.0 (9.0–10.0)	0.173
Apgar min 10	10.0 (10.0–10.0)	10.0 (10.0–10.0)	0.280
5-min Apgar < 7	4 (7.2)	2 (3.6)	0.388
Admission to NICU	2 (3.6)	3 (5.4)	0.647
Arterial cord blood analysis results			
pH	7.25(0.09)	7.28 (0.08)	0.137
Umbilical artery pH ≤ 7.15	5 (9.1)	3 (5.4)	0.430
Base excess, mmol/l	-5.4 (3.4)	-4.9 (3.0)	0.431
pO_2, mmHg	21.1 (17.0–26.2)	17.9 (13.0–23.0)	0.035*
pCO_2, mmHg	51.2 (11.1)	50.7 (9.4)	0.826
Lactate mmol/l	4.1 (1.4)	4.0 (1.6)	0.861
Glucose, mg/dl	81.7 (20.8)	75.5 (19.0)	0.187

Data are median (interquartile range) or mean (SD) or *n* (%)

PSS perceived stress scale, *BMI* body-mass index, *NICU* neonatal intensive care unit

*$p < 0.05$

[a]Missing data of 14 CG and 17 SG; 0: control group (PSS < 19) 1: stress group (PSS ≥ 19)

There is no recommended cut-point for high stress, and other studies have stratified this continuous variable using study-specific thresholds between 67 and 75% [3, 26]. In our case, we performed a pilot study and we found that the 80% quantile, to be on the safe side, of the PSS-10 was 19, which was then used as the cut-off score for stressed (SG) and control group (CG) in this study, also in accordance to prior studies [3, 26]. PSS-score ≥ 19 corresponded to 22% women in our pilot study, another 22% showed PSS-10 score < 10 and 56% had a PSS-10 score between 10 and 19.

Phase II: Maternal and fetal prenatal ANS assessment

Prospective participants attended an informational session, where procedures were explained in detail, formal enrollment completed, and the consent forms from the participants obtained. We collected demographic information from the consented women.

Two and a half weeks after screening, we performed a transabdominal ECG (taECG) recording at 900 Hz sampling rate of at least 40 min duration using AN24 (GE HC/Monica Health Care, Nottingham, UK).

We applied the fetal ECG extraction algorithm SAVER [27] to detect the fetal R-peaks and the maternal R-peaks in the taECG separately. With the fetal- and maternal R-peaks, we obtained the fetal- and maternal RR interval time-series. The quality of taECG was estimated by the calculation of signal quality index (SQI) within windows of one second each. Regions where SQI was lower than 0.5 were marked as artifacts and were not considered for the analysis. Mean fetal heart rate (fMHR) and mean maternal heart rate (mMHR) were calculated. Mean maternal respiratory rate was derived from taECG and calculated according to Sinnecker et al. [28].

Phase III: Delivery

Newborn recordings: Clinical data including birth weight, length and head circumference, pH and Apgar score, were recorded.

Maternal cortisol assessment: On the day of parturition, hair strands (~3 mm diameter) were collected from the posterior vertex region on the head as close to the scalp as possible [29]. Hair samples were sent to the Department of Clinical Biochemistry (Endocrinology Section) of the Faculty of Pharmacy and Biochemistry (University of Buenos Aires) for cortisol measurement using auto-analyzers. Based on an approximate hair growth rate of 1 cm per month, the proximal 3 cm long hair segment is assumed to reflect the integrated hormone secretion over the three-month-period prior to sampling. The 3 cm hair sample was wrapped in aluminum foil for protection and stored at room temperature up to three months. For the transatlantic air transport, aluminum stored hair samples were additionally and individually wrapped in insulation material during the flight to ensure to keep the temperature as stable as possible. For analysis, fifty milligrams of hair obtained from the 3 cm closest to the roots (equivalent to 3 months of growth), were weighed in an analytical balance, as recommended by the Society of Hair Testing [29]. The cortisol was extracted and measured according to Iglesias et al. [30]. This procedure has been validated with the standard method of mass spectrometry and was patented by the University of Buenos Aires [31].

Bivariate phase-rectified signal averaging (BPRSA)

The bivariate PRSA method is an extension of the "monovariate" PRSA method that we introduced for the analysis of fetal heart rate [32, 33].

Bivariate phase-rectified signal averaging allows for identifying and quantifying relationships between two synchronously recorded signals [17]. In this study these two signals are the maternal heart rate (mHR) as the trigger signal and the fetal heart rate (fHR) as the target signal.

The algorithm of BPRSA consist of four steps:

1. At the beginning we identify all decreases in mHR and mark them as so called anchor points A.
2. To investigate the response of the fetus at the defined anchor points the fHR is interpolated with a sample rate of 900 Hz as maternal ECG is registered with 900 Hz. Anchor points are identified by the time of occurrence within the fHR and are denoted as A'.

   Time frames of a certain length ("2L") around each anchor A' are selected in the fHR signal. In the current study we used $L = 9000$ samples, which corresponds to a window of 20 s.
3. All segments are aligned at the anchors leading to a phase-rectification of the segments. The BPRSA-signal X is obtained by averaging the aligned segments. Deflections in the BPRSA-signal can be interpreted as coupling between mHR and fHR.
4. Finally, the BPRSA-signal X is quantified within a defined time span prior to and after the center of X. The time span starts 1.5 s after the center and ends at 2.5 s after the center. Therefor we use $S1$ and $S2$ as additional indices for the quantification. The data frame after the center is defined as $L+S1$ up to $L+S2$. On the other side, the data frame prior to the center is defined as $L-S2$ up to $L-S1$. Using $S1 = 1350$ and $S2 = 2250$ reflects the sample rate of 900 Hz in the BPRSA signal X and corresponds to 1.5 s and 2.5 s, respectively. Coupling between mHR and fHR was analyzed by BPRSA resulting in a new parameter called fetal stress index (FSI). FSI is quantified by calculating the difference

between the mean values of the data frames after and prior the center of X.

$$\mathrm{FSI} = \frac{1}{S2 - S1} \sum_{i=L+S1}^{L+S2} X(i) - \frac{1}{S2 - S1} \sum_{i=L-S2}^{L-S1} X(i)$$

Note that the center of X (at indices L) corresponds to our anchor definition that was performed within the mRRI. Thus, the FSI measures the response of the fetus on maternal heart rate decreases.

Statistics

Normal distribution was tested using Shapiro–Wilk test. For skewed distribution, medians and interquartile ranges were reported, and for Gaussian distribution, mean and standard deviation. For categorical data, we show the absolute and relative frequencies. For comparison between groups, Mann–Whitney U tests, t test for independent samples and Pearson Chi-squared test were used. Receiver operating characteristics (ROC) analyses were performed to estimate the predictive performance of the quantitative variables for the presence of PS.

For each fetus and mother, the fHR and mHR recorded at the same time were analyzed.

Finally, Pearson's correlation coefficient was used to evaluate the relationship between the fHR and mHR. All statistical tests were conducted two-sided and a p value < 0.05 was considered statistically significant for all comparisons. The fetal HR extraction algorithm was carried out in MatlabR2016a. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

Study approval and funding

The study protocol is in strict accordance with the Committee of Ethical Principles for Medical Research from TUM and has the approval of the "Ethikkommission der Fakultät für Medizin der Technischen Universität München" (registration number 151/16S). ClinicalTrials.gov registration number is NCT03389178. Written informed consent was received from participants prior to inclusion in the study. The project was developed and performed by own resources of Frauenklinik/Klinikum rechts der Isar.

Results

Sociodemographic parameters and perinatal outcomes

Between July 2016 and May 2018 of all 1500 screened women, 538 (35.8%) returned the questionnaire and 89/538 (16.5%) scored ≥ 19 on PSS-10 classifying as SG (Figure S1). Based on recruitment criteria 55/538 (10.2%) subjects were included in the stress group (SG) of the study and the control group (CG) comprised 55 of possible 449 (12.2%) subjects at a similar median gestational age of 34.0 weeks. The cohort characteristics and perinatal outcome variables are summarized in Table 1.

Median PSS of the SG was 22.0 (1st—3rd quartile: 21.0–24.0) and that of the CG9.0 (6.0–12.0) ($p < 0.001$), respectively. The cortisol in maternal hair was 63% higher in SG versus CG ($p = 0.029$) confirming the PSS results. The correlation of PSS and cortisol in hair was 0.182, no statistically significant association was observed ($p = 0.098$). Area under the ROC curve for hair cortisol (prediction PS) was 0.639 ($p = 0.029$).

The pregestational [24.2 (20.9–30.8) versus 21.5 (20.2–23.5), $p = 0.001$] and at-study-inclusion [29.8 (26.0–36.7) versus 26.1 (24.5–28.7), $p = 0.001$] median BMI of the SG patients was higher. Cord blood arterial pO_2 was lower in the SG fetuses [17.9 (13.0–23.0) versus 21.1 (17.0–26.2) mmHg, $p = 0.035$].

There were 9 times as many women in the SG diagnosed with gestational diabetes mellitus (GDM) than in the CG ($p = 0.008$) and 5 times as many diagnosed with cases of autoimmune diseases ($p = 0.014$).

Smoking was more frequent in the SG ($p = 0.028$) and the number of planned pregnancies was lower in the SG than in the CG ($p = 0.001$).

Less SG subjects visited university ($p = 0.001$) and had the monthly net-income per household above 5000€ ($p = 0.002$). More than two times as many SG than CG subjects ended up in cesarean delivery ($p = 0.007$). The rate of cesarean deliveries after onset of labor was similar in both groups but 25.4% had a "planned" cesarean delivery being in the SG versus 5.4% of the women being in the CG. Indications for the "planned" cesarean delivery in the SG were breech presentation, prior cesarean delivery, maternal indication and placenta praevia. In the CG the only indication was prior cesarean delivery.

BPRSA

Data from 104 out of 110 subjects were used, because 6 subjects had poor ECG signal quality (2 CG fetuses and 4 SG).

Mean mHR, mean maternal-respiratory rate calculated from Mecg, and median fHR were similar in both groups. Median FSI was significantly higher in SG compared to controls [0.43 (0.18–0.85) versus 0.00 (−0.49–0.18), $p < 0.001$] (Fig. 1). This means that SG fetuses showed fHR decreases whereas CG fetuses remained similar after the maternal anchor "mHR decreases". This difference remained significant even after adjustment of relevant socioeconomic

differences between both groups (BMI, university degree, household income > 5000€/month, smoking, planned pregnancy, diabetes, autoimmune diseases; $p = 0.012$). Area under the receiver operating characteristics curve was 0.748 ($p < 0.001$) (Fig. 2). Figure 3 illustrates BPRSA results showing the fetal response to maternal heart rate decreases.

Apart, we found a significant correlation of PSS and FSI ($r = 0.34$, $p < 0.001$).

Discussion

In this pilot study, we show that prenatal maternal stress identified in the third trimester by a validated questionnaire (PSS-10) shows a correlation with the coordination of fetal- and maternal heart-rate and fetal oxygenation at birth. The proposed BPRSA index (FSI) provides unique insights into the relationship between two biological systems, mother and fetus. We could detect periodic mHR decreases reflecting typical pattern of maternal breathing (sinus bradycardia during expiration). Interestingly, CG fetuses remained "stable" during these periods whereas fetuses of stressed mothers showed significant decreases of fHR. We hypothesize that this response is induced by the mechanical stimuli (diaphragm excursion that changes the uterine pressure). It is well known that maternal anxiety and stress can evoke immediate changes in uterine blood flow, fetal heart rate (FHR) or fetal movements (FM), and induce long-term changes in fetal growth, metabolism, behavior and cognition.

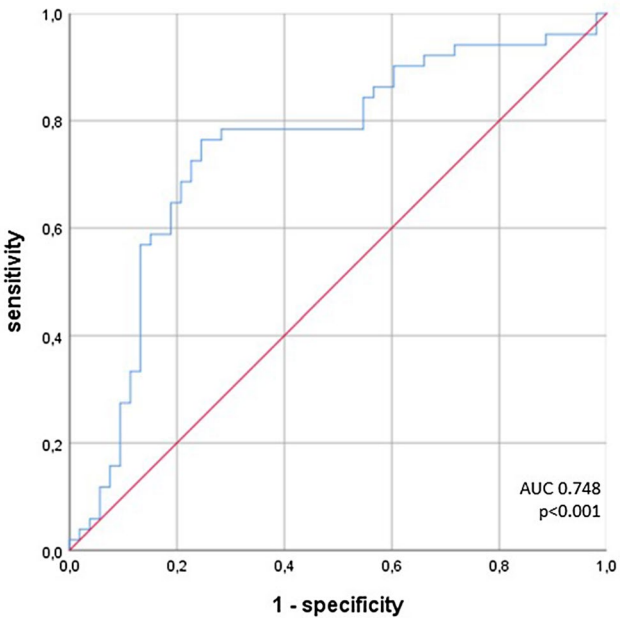

Fig. 2 Area under the receiver operating characteristics curve for prediction of maternal stress using bivariate phase-rectified signal averaging method

Future studies will need to address the question why CG fHR remained unchanged whereas SG showed decreased

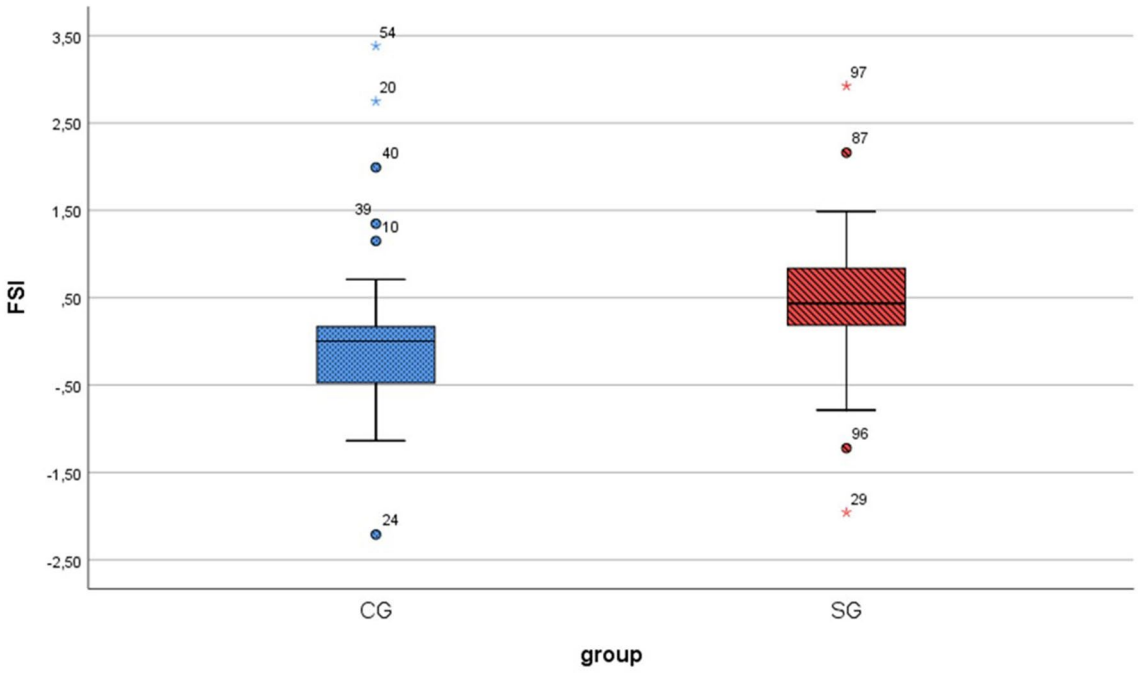

Fig. 1 Box plots using bivariate phase-rectified signal averaging (BPRSA) method comparing controls (CG, blue, dotted) and stress group (SG, red, striped)

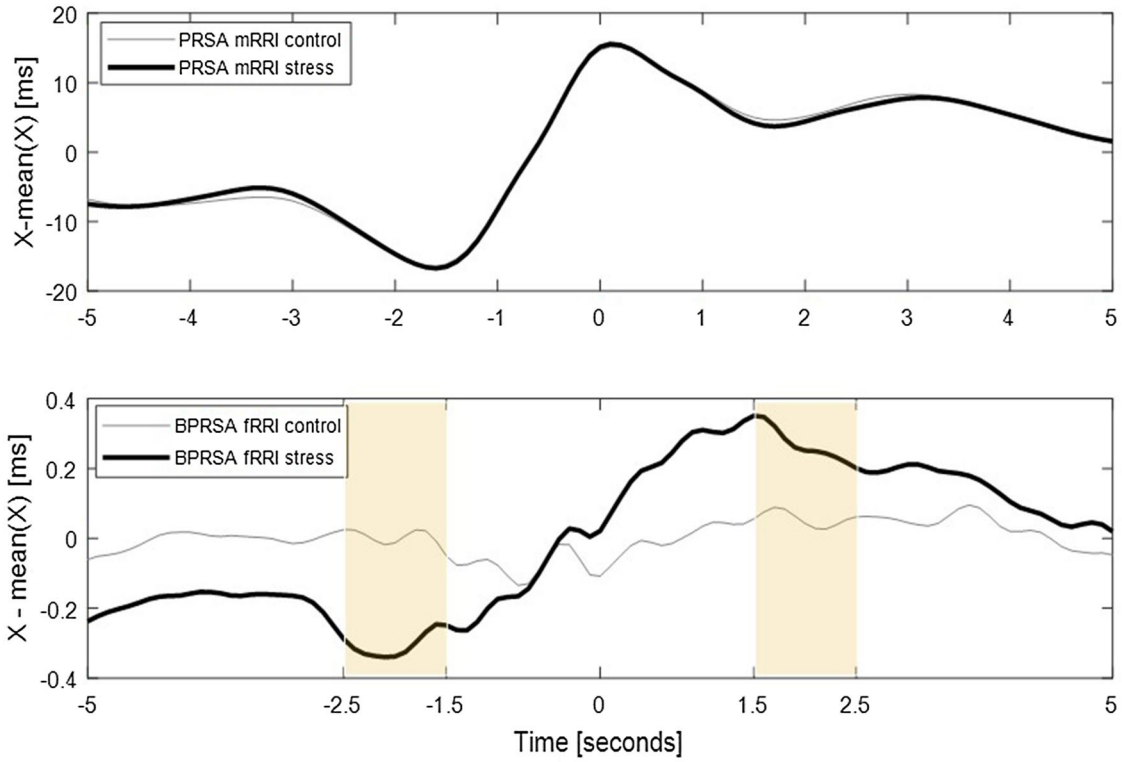

Fig. 3 Bivariate phase-rectified signal averaging (BPRSA) analysis of RR intervals. (Top) PRSA signal *X* for maternal RR intervals (mRRI). The anchor point definition, namely all heart rate deceleration, reflect the central oscillation of *X*. (Bottom) the response of fetal RR intervals (fRRI) on the maternal decreases. The signal of the control BPRSA shows a significant lower response than the BPRSA curve for the fetus of a stressed mother. Also shown is the time span which is used for the quantification of fetal stress index (FSI) (yellow)

fHR during maternal breathing. A possible explanation might be the "over-sensitization" of SG fetuses' HPA axis or the differences in maturation of the sympathetic and parasympathetic branches of the ANS in contrast to the CG similar to data derived from animal models where lower-weight twin sheep fetuses showed increased sympathetic activity and immaturity of circulatory control [34]. Physiologically, the ability of two-complex weakly coupled systems to entrain each other is influenced by their intrinsic oscillatory properties and maturation, respectively. A well-studied example can be found in the cardiac pacemaker physiology [35].

Fetal ANS is very sensitive to maternal stress [36–38] and common markers of ANS such as fHR reactivity to a stimulus, reflect emerging individual differences in the development of the autonomic- and central nervous systems related to styles of future emotional regulation and risk for psychopathology [39, 40]. It is hence likely that the fHR response to mHR changes represents a fetal stress memory and may serve as a novel biomarker to detect PS effects early in utero which may help guide early interventions postnatally. For example, PS was linked to increased risk for autism spectrum disorder and alterations of ANS in autism spectrum disorders children have been reported [41, 42].

We observed a mild fetal hypoxia at birth compatible with the concept of chronic reduction of uterine blood flow due to PS [43]. The result may be a reduced placental-catecholamine clearance, thus elevating fetal catecholamine levels with hyperactive HPA axis and sympathetic nervous system. The postnatal developmental sequelae of these adaptations remain to be elucidated.

The persistent exposure to stress is validated on the maternal side by the higher maternal-chronic cortisol levels at delivery in SG compared to CG. Perceived (subjective) measures of maternal stress did not correlate well with maternal hair cortisol in our study. This agrees with the body of inconclusive evidence seeking to link subjective stress exposure with cortisol values [30, 44–46]. The most likely cause of the lack of correlation is the presence of mechanisms mediating PS effects that do not alter maternal cortisol levels. As reviewed by Rakers et al. [4], there are further potential mediators that connect the stressed mother with the fetus besides cortisol such as catecholamines, reactive oxygen species, cytokines, serotonin/tryptophan, and maternal microbiota, similarly reflecting the autonomic nervous system. fHR changes may be a good biomarker mediating PS-effects as evidenced by our finding that FSI was more predictive of the subjective stress perception than cortisol.

This result is in line with the psychological body of work such as the polyvagal theory [47] where heart-rate variability has served as a good biomarker of internal emotional regulation which, in turn, reflects upon subjective coping with daily stress.

Our study has several strengths and limitations. One of the strengths is the prospective observational study design including women experiencing daily hassles stress rather than extreme stress exposures. We believe this makes the findings generalizable onto population of pregnant women seen in most antepartum follow-up centers. Our demographic data indicated distinct features of SG and CG regarding metabolic- and socioeconomic status. This may be seen as a confounding factor for FSI, but even after adjustment for these possible confounders, the difference of FSI between both groups remains significant. When planning the study we decided to match only for known influence factors on fetal ANS (gestational age, maternal age) and birth outcome (parity). After having performed the analysis we obtained significant differences in socioeconomic parameter as university degree, income, smoking status, BMI, autoimmune disease, and GDM reflecting parameter which might be the cause of the maternal stress or at least playing a role for stress therefore being significantly more frequent in our SG. We interpret these findings as a possible source for maternal stress rather than a random difference of the study group. The best scenario would have been to include the whole cohort However, since all women qualifying as SG were enrolled, but not all controls, we suggest this had no impact on the reported differences due to PS.

With an objective and low-cost biomarker of PS impact on mother and fetus in hand, prevention should be the subject of the future studies. Given that psychometric instruments by itself have been shown to be insufficient and inconsistent to diagnose prenatal stress, González-Ochoa et al. [48] recently proposed that a combination of clinical, physiological, and biochemical studies might improve the precision of stress assessment in gestant mothers to involve children in early stimulation programs and parenting support. In view of this proposal, we envision that FSI might become a powerful objective measure of the effects on stress on maternal ANS as well as in the transgenerational influence on fetal development.

Our findings warrant further investigations into the mechanisms of maternal–fetal stress transfer under the widely prevalent conditions of daily hassles, identification of epigenetic biomarkers impacting the stress axis and ANS activity and postnatal consequences of intrauterine imprinting of maternal stress upon fetal physiology.

In conclusion, we validated our hypothesis that PS-induced programming is reflected in mHR- and fHR biomarkers of ANS activity. The biomarkers we identified can be harnessed for early detection and follow-up of children affected by PS. Early detection of altered neurodevelopmental trajectories opens new possibilities for designing more timely and effective interventions to improve outcomes of pregnancy affected by PS.

Acknowledgements MCA was awarded with the August Wilhelm Scheer Professorship Program (TUM) twice for a 6 months period stay at the Klinik und Poliklinik für Frauenheilkunde, Technische Universität München, Klinikum rechts der Isar, Munich for the start-up of the FELICITy project and a Hans Fischer Senior Fellowship from IAS-TUM (Institute for Advanced Study-TUM, Munich).

Author contributions SML and MCA: protocol and project development, data collection and management, data analysis, and manuscript writing and editing. MF: data collection management, data analysis, and manuscript writing and editing. CZ and JW: patient recruitment, data collection, and manuscript writing and editing. AM and GS: data analysis, manuscript writing and editing. BH: statistical analysis and advice, manuscript writing and editing. CS and PCS: machine learning analysis, manuscript editing. HTW: machine learning analysis and manuscript writing and editing. BF and GB: cortisol analysis in hair samples, manuscript writing and editing.

Compliance with ethical standards

Conflict of interest HTW and MGF hold a provisional and a PCT patent on fetal ECG technology.

Ethical approval The study protocol is in strict accordance with the Committee of Ethical Principles for Medical Research from TUM and has the approval of the "Ethikkommission der Fakultät für Medizin der Technischen Universität München" (registration number 151/16S). ClinicalTrials.gov registration number is NCT03389178. Written informed consent was received from participants prior to inclusion in the study.

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Affiliations

Silvia M. Lobmaier[1] · A. Müller[2] · C. Zelgert[1] · C. Shen[3] · P. C. Su[3] · G. Schmidt[2] · B. Haller[4] · G. Berg[5] · B. Fabre[6] · J. Weyrich[1] · H. T. Wu[7,8,9] · M. G. Frasch[10] · M. C. Antonelli[1,11]

A. Müller
alexander.mueller@mytum.de

C. Zelgert
camilla.zelgert@web.de

C. Shen
chao.shen@duke.edu

P. C. Su
b94401079@gmail.com

G. Schmidt
gschmidt@tum.de

B. Haller
bernhard.haller@tum.de

G. Berg
aberg@ffyb.uba.ar

B. Fabre
brfabre2000@yahoo.com.ar

J. Weyrich
joy.weyrich@yahoo.de

H. T. Wu
hauwu@math.duke.edu

M. G. Frasch
mfrasch@uw.edu

M. C. Antonelli
mca@fmed.uba.ar

[1] Department of Obstetrics and Gynecology, Klinikum Rechts Der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany

[2] Innere Medizin I, Department of Cardiology, Klinikum Rechts Der Isar, Technical University of Munich, Munich, Germany

[3] Department of Mathematics, Duke University, Durham, NC 27705, USA

[4] Institute of Medical Informatics, Statistics and Epidemiology, Buenos Aires, Argentina

[5] Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas Y Técnicas (CONICET), Facultad de Farmacia Y Bioquímica, Buenos Aires, Argentina

[6] Facultad de Farmacia Y Bioquímica. Instituto de Fisiopatología Y Bioquímica Clínica (INFIBIOC), Universidad de Buenos Aires, Buenos Aires, Argentina

[7] Department of Mathematics, Duke University, Durham, NC 27705, USA

[8] Department of Statistical Science, Duke University, Durham, NC 27705, USA

[9] Mathematics Division, National Center for Theoretical Sciences, Taipei, Taiwan

[10] Department of Obstetrics and Gynecology, University of Washington, Seattle, WA, USA

[11] Instituto de Biología Celular Y Neurociencia "Prof. E. De Robertis", Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina