

# Cumulative Life Stressors and Stress Response to Threatened Preterm Labor as Birth Date Predictors

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## Research Article

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# Abstract

**Purpose:** Preterm birth represents one of the main causes of neonatal morbimortality and a risk factor for neurodevelopmental disorders. Appropriate predictive methods for preterm birth outcome, which consequently would facilitate preventing programs, are needed. We aim to predict delivery date in women with a threatened preterm labor (TPL) based on stress response to TPL diagnosis, cumulative life stressors, and relevant obstetric variables.

**Methods:** A prospective cohort of 157 pregnant women with TPL diagnosis between 24 and 31 weeks gestation formed the study sample. To estimate the stress response to TPL, maternal salivary cortisol,  $\alpha$ -amylase levels, along with anxiety and depression symptoms were measured. To determine cumulative life stressors, previous traumas, social support, and family functioning were registered. Then, linear regression models were used to examine the effect of potential predictors of birth date.

**Results:** The main predictors were lower family adaptation, higher Body Mass Index (BMI), higher cortisol levels and TPL diagnosis week, which showed a non-linear interaction with cortisol levels: TPL women with middle- and high-cortisol levels before 29 weeks of gestation presented an imminent labor.

**Conclusion:** A combination of stress response to TPL diagnosis (salivary cortisol) and cumulative stressors (family adaptation) together with obstetric factors (TPL week and BMI) was the best birth date predictor. Therefore, a psychosocial therapeutic intervention program aimed to increase family adaptation and decrease cortisol levels at TPL diagnosis as well as losing weight, may prevent preterm birth in symptomatic women.

## Introduction

Despite advances in increasing survival rate, preterm birth is still the main cause of neonatal morbidity [1], representing one of the most leading risk factors for neurodevelopmental disabilities during childhood [2]. Critically, the lack of accurate prediction methods of preterm birth in TPL women is a matter of concern due to the potential iatrogenic effects of repeated antenatal corticosteroid on the future child's neurodevelopment [3, 4], stressful and unnecessary hospitalizations, and elevated costs for public health system [5, 6]. One-third of hospitalized pregnant women suffer from a threatened preterm labor (TPL), but more than 50% do not progress to active labor [7] and only about half of preterm births are preceded by a known risk factor [8]. Different prevention programs have been implemented around the world aimed to reduce preterm birth [9] and social determinants are gaining consideration from scientific organizations [10]. Therefore, reliable methods to stratify TPL women into low and high-risk groups for preterm birth outcome are required.

A growing body of research has indicated that both chronic life stress prior to conception and stressful events during pregnancy may act as potential risk factors for preterm birth [11, 12]. When coping mechanisms are saturated due to chronic life stress, overexposure to neuroendocrine mediators (e.g., cortisol or  $\alpha$ -amylase) that maintain the homeostasis of Hypothalamic-Pituitary-Adrenal (HPA) axis [13,

14] and the Sympathetic-Adrenal-Medullary (SAM) axis [14] may have a deleterious impact on both mother and fetus [15], increasing the risk of preterm birth. Among possible causes of chronic stress, a history of traumatic events and poor social or family functioning have usually been identified. In fact, it is well-documented that a history of traumatic life events prior to conception may increase the risk of preterm birth [16–20]. However, although social support may modulate the association between life stressful events and preterm birth, findings are inconclusive [12, 21–23]. Whereas a systematic review concluded null relationship between maternal social support and preterm birth [22], more recent studies pointed out that the lack of partner support rather than lack of general social support was associated with higher risk of preterm birth [21, 23, 24].

Regarding stressful events during pregnancy, they may alter normal balance of immune mediators, hormones, and neurotransmitters involved in timing of birth, increasing the risk of preterm birth [25, 26] as well as psychomotor impairments [27]. Noteworthy, TPL is considered a stressful prenatal event likely to trigger a biopsychological stress response [28a, 28b]. First, from a biological perspective, TPL event may dysregulate both the HPA axis [13, 14] and the SAM axis [14]. As for HPA axis biomarkers, research has revealed that cortisol levels at TPL diagnosis may predict birth 48 hours after TPL diagnosis [29]. Conversely, other study addressing SAM activity measured by  $\alpha$ -amylase levels has showed inconclusive findings [14]. Whereas  $\alpha$ -amylase dysregulation has been suggested as the underlying mechanism for the link between maternal depression and prematurity [30], no association between  $\alpha$ -amylase levels and preterm birth has been found in non-depressed women [28a, 28b]. Second, from a psychological perspective, both gestational anxiety [31, 32] and depressive symptoms [33–35] which may be triggered by TPL diagnosis [36] can also be associated with preterm birth. In sum, women experiencing chronic life stress may need only another significant stressor during pregnancy such as TPL to reach the tipping point that leads to a preterm birth [31].

In spite of focused research efforts, it is still unclear which stress-related factors are most strongly associated with preterm birth, for several reasons. Firstly, although the impact of a stressful event during pregnancy can be modulated by a combination of biopsychosocial stress-related pathways (HPA or SAM stress biomarkers, anxious-depressive symptoms at TPL diagnosis, and/or previous traumatic events as well as social support), most studies have considered these pathways separately [37]. Secondly, the relationship between these biomarkers and self-reported psychosocial stress is not straightforward [38]. Moreover, the few studies that have simultaneously examined different stress-related outcomes have included asymptomatic pregnant women (i.e., without TPL), reporting inconclusive findings: whereas some studies not using self-reports found an association between preterm birth and stress biomarkers [39–40], others found that maternal self-reports may improve the biomarkers prediction [41]. Thirdly, prospective studies with symptomatic women usually conduct a follow-up until 48 hours after a TPL diagnosis instead of until birth [29]. In the present follow-up study, multiple stress-related outcomes are studied simultaneously in symptomatic women from TPL diagnosis to birth date.

This study aims to predict the birth date in TPL women by means of a combination of multiple stress-related factors: (i) cumulated life stressors (previous traumas, social support, and family functioning);

and (ii) biopsychological response to TPL diagnosis (salivary cortisol and  $\alpha$ -amylase as well as anxiety and depression symptoms). We expect that, considering studies that examine a combination of stress-related variables, biomarkers would be the strongest birth date predictors [39–40]. However, self-reports assessing chronic social stress and psychological stress response to TPL diagnosis may improve this prediction [41]. Also, the association between biomarkers and self-reports should not be constrained to be linear [38].

## Material And Methods

This is a prospective cohort study performed in the Division of Obstetrics at a tertiary referral hospital during a 12-month period. The Ethics Committee at the Health Research Institute approved the study protocol (ref. 2015/0086) and informed consent was obtained from all participants.

## Participants

Eligible participants were pregnant women diagnosed of TPL between 24- and 31 + 6- weeks gestation to guarantee that all participants were subjected to the same treatment. TPL was diagnosed if the following clinical signs were present: regular uterine contractions associated with cervical changes ( $\geq 80\%$  cervical effacement or cervical dilation  $\geq 2$  cm), measured by the cervical ultrasound (cervical length  $< 25$  mm). After TPL diagnosis, fetal cardiac activity and uterine contractions were monitored by abdominal ultrasound. If contractions continued, women were admitted to the obstetric ward [42]. All women received one corticosteroids dose at least 12 hours before saliva sample collection, and the second corticosteroids dose was administered after it. Considering that corticosteroid average lifetime is 12 hours, antenatal steroid levels decreased notably when saliva sample was collected. Tocolytic therapy was atosiban or nifedipine [43]. Atosiban was initiated with a 6.75 mg/min bolus. Then, 300  $\mu\text{g}/\text{min}^{-1}$  as loading dose for 3 hours and 100  $\mu\text{g}/\text{min}^{-1}$  as maintenance dose for 48 hours was administered. Alternatively, nifedipine 20 mg, followed by 10 mg each 20 minutes until 40 mg for 1 hour, was administered [44]. Thus, all women received atosiban or nifedipine for  $< 24$  hours. Finally, in cases of imminent labor (cervix between 4–10 cm dilated, rate of cervical dilation at least 1 cm/hour, effacement is usually complete, and fetal descent through birth canal begins), magnesium sulfate is usually administered but, in our sample, none of the participants received magnesium sulfate before saliva sample collection.

Exclusion criteria included severe medical conditions (e.g., diabetes mellitus), severe obstetric complications (placenta abruption, preeclampsia, intrauterine growth restriction, cervical dilation  $> 4$  cm, infection, obstructed labor), fetal anomalies, teratogenic substances use, and social exclusion risk, which is considered a stressful condition that may act as confusing variable. To assess social exclusion risk, multidimensional criteria were employed: (i) risk of poverty; (ii) severe material deprivation; and/or (iii) jobless household [45]. A final sample of 151 TPL women completed the follow-up until birth. See Fig. 1 for the recruitment flow diagram.

# Instruments and procedure

*Psychological assessment.* The following questionnaires were completed by participants in a 1 hour-session following recruitment.

- The Traumatic Experience Questionnaire (TEQ) [46] is a screening test to diagnose post-traumatic stress disorder. The questionnaire reports in three parts: (i) list of traumatic experiences; (ii) the most important traumatic event; and (iii) list of symptoms; whose sum represents the total score.
- The Multidimensional Scale of Perceived Social Support (MSPSS) [47] assesses an individual's perception of the social support from family, friends, and significant others (partner).
- The Family Adaptability Cohesion Evaluation Scale III (FACES III) [48] measures family Cohesion (degree to which family members are separated from or connected to their family) and family Adaptability (extent to which the family system is flexible and able to change facing new circumstances).
- The State-Trait Anxiety Inventory (STAI) [49] assesses trait and state anxiety. It can be used in clinical settings to diagnose anxiety and to distinguish it from depressive syndromes. Higher scores indicate greater anxiety.
- The Beck Depression Inventory Short Form (BDI/SF) [50] measures characteristic attitudes and symptoms of depression for psychiatric and non-psychiatric populations.

*Stress biomarkers.* Concerning analytical determinations, standard of cortisol was purchased from Sigma-Aldrich Química SA (Madrid, Spain). Saliva samples were collected on the morning after admission between 10–12 a.m. (minimum 1h after breakfast). Samples were stored at -80 °C and were thawed on ice and homogenized. The sample treatment to determine cortisol was based on a previous work [51]. Briefly, 25 µL of sample were subjected to liquid-liquid extraction to extract cortisol, then the organic layer was evaporated to dryness and the residues were reconstituted in water (pH 3): methanol (85:15 v/v) solution. Finally, 5 µL were injected in the chromatographic system (ultra-performance liquid chromatography coupled to tandem mass spectrometry).

Salivary  $\alpha$ -amylase assay kit was acquired from Salimetrics (Suffolk, United Kingdom). For the  $\alpha$ -amylase determination, samples were vortexed and centrifuged. Then, they were diluted with the  $\alpha$ -amylase diluent at 1:200 as final dilution. Finally, they were subjected to the kinetic enzyme assay.

## Statistical analysis

As for statistical analysis, data were summarized using mean (standard deviation) and median (1st, 3rd quartile) for continuous variables and relative and absolute frequencies for categorical variables. Correlations among stress-related variables were assessed with Spearman's correlation. Association between potential predictors and birth week was assessed using a linear regression model. Parity, Body Mass Index (BMI), multiple pregnancy, *in-vitro* fertilization, and the TPL diagnosis week were also included due to their potential influence on preterm birth [52]. Selection of the predictors included in the

model was performed using L1 penalization. The lambda parameter was selected using 500 repetitions of 10-fold cross-validation. Model performance was assessed estimating optimism corrected R-square using bootstrapping [53]. All statistical analyses were performed using R (version 3.5.3), rms (version 5.1–3.1) and glmnet (version 2.0–16).

## Results

Socio-demographic and clinical variables of the participants are summarized in Table 1. Prior to modelling, an exploratory data analysis was performed by examining correlations between the different stress-related variables (Fig. 2). Chronic stress-related variables showed moderate to strong correlations among them (MSPSS and FACES). Similarly, psychological stress-related responses to TPL (STAI and BDI) moderately correlated to each other. No other evident associations were found.

Table 1  
Sociodemographic and clinical variables of the final sample.

| Variable                      | Final sample<br>(n = 157) |
|-------------------------------|---------------------------|
|                               | Mean (SD) / n(%)          |
|                               | Median (1st, 3rd Q.)      |
| Maternal age                  | 31.75 (5.41)              |
|                               | 32 (28, 36)               |
| Parity                        | 0.52 (0.92)               |
|                               | 0 (0, 1)                  |
| BMI                           | 22.58 (3.07)              |
|                               | 22.04 (21, 23)            |
| Threatened preterm labor week | 29.57 (2.87)              |
|                               | 30 (28, 32)               |
| State STAI                    | 20.01 (9.72)              |
|                               | 18 (14, 24)               |
| Trait STAIR                   | 17.86 (8.76)              |
|                               | 17 (11, 22)               |
| BDI – II                      | 3.01 (3.11)               |
|                               | 2 (1, 4)                  |
| Friends MSPSS                 | 25.18 (3.61)              |
|                               | 27 (24, 28)               |
| Family MSPSS                  | 26.43 (2.6)               |
|                               | 28 (26, 28)               |
| Partner MSPSS                 | 27.1 (1.91)               |
|                               | 28 (27, 28)               |
| Adaptation FACES III          | 30.53 (6.42)              |
|                               | 30 (28, 34)               |

BMI: Body Mass Index; STAI: State-Trait Anxiety Inventory; BDI/SF: Beck Depression Inventory Short Form; MSPSS: Multidimensional Scale of Perceived Social Support; FACES: Family Adaptability Cohesion Evaluation Scale; TEQ: Traumatic Experience Questionnaire.

| Variable   | Final sample<br>(n = 157) |
|--|---------------------------|
| Cohesion FACES III   | 31.73 (5.32)              |
|  | 32 (29, 35)               |
| TEQ  | 2.91 (4.26)               |
|  | 0 (0, 5)                  |
| Cortisol (nmol L <sup>-1</sup> )   | 3.06 (4.95)               |
|  | 1.33 (0.05, 3.61)         |
| α-amylase (U mL <sup>-1</sup> )  | 68.18 (65.42)             |
|  | 54.12 (27.95, 78.92)      |
| <i>In-vitro</i> fertilization  |                           |
| No   | 141 (89.81%)              |
| Yes  | 16 (10.19%)               |
| Multiple pregnancy   |                           |
| No   | 89 (56.69%)               |
| Yes  | 68 (43.31%)               |
| BMI: Body Mass Index; STAI: State-Trait Anxiety Inventory; BDI/SF: Beck Depression Inventory Short Form; MSPSS: Multidimensional Scale of Perceived Social Support; FACES: Family Adaptability Cohesion Evaluation Scale; TEQ: Traumatic Experience Questionnaire. |                           |

Variable selection using L1 penalization specified four predictors as the optimum complexity for the linear regression predictive model (Table 2). These variables were family Adaptation (FACES), BMI, TPL week, and cortisol levels. Additionally, a non-linear trend for TPL week using regression splines was added to the model.

Table 2  
Results of the fitted linear regression model to predict the birth week.

| Variable   | Coefficient | 95% CI         | p-value |
|--|-------------|----------------|---------|
| (Intercept)  | -43.49      | [-70.3, -16.7] | 0.002   |
| Adaptation FACES III   | 0.11        | [0.026, 0.19]  | 0.01    |
| BMI  | -0.23       | [-0.41, -0.05] | 0.012   |
| log(cortisol)  | -18.21      | [-28.9, -7.54] | 0.001   |
| TPLweek  | 2.92        | [1.91, 3.92]   | < 0.001 |
| TPLweek'   | -3.04       | [-4.53, -1.55] | < 0.001 |
| TPLweek''  | 17.27       | [6.43, 28.1]   | 0.002   |
| TPLweek:log(cortisol)  | 0.66        | [0.25, 1.07]   | 0.002   |
| TPLweek':log(cortisol)   | -0.75       | [-1.38, -0.11] | 0.022   |
| TPLweek'':log(cortisol)  | 4.91        | [0.19, 9.63]   | 0.041   |
| BMI: Body Mass Index; FACES: Family Adaptability Cohesion Evaluation Scale; TPL: Threatened Preterm Labor. |             |                |         |

$$\text{Birth Week} = -43.49 - 0.23 \cdot \text{BMI} + 2.92 \cdot \text{TPLweek} - 0.04 \cdot \max(\text{TPLweek} - 24, 0)^3 + 0.21 \cdot \max(\text{TPLweek} - 29, 0)^3 - 0.26 \cdot \max(\text{TPLweek} - 31, 0)^3 + 0.009 \cdot \max(\text{TPLweek} - 33, 0)^3 - 18.21 \cdot \log(\text{cortisol}) + 0.11 \cdot \text{adaptation} + \log(\text{cortisol}) \cdot (0.66 \cdot \text{TPLweek} - 0.009 \cdot \max(\text{TPLweek} - 24, 0)^3 + 0.06 \cdot \max(\text{TPLweek} - 29, 0)^3 - 0.08 \cdot \max(\text{TPLweek} - 31, 0)^3 + 0.03 \cdot \max(\text{TPLweek} - 33, 0)^3)$$

This model had an R-squared value of 0.37 and an optimism corrected R-squared value of 0.30. To aid in the interpretation of the non-linear effect of TPL week, a marginal effects plot for this variable and its interaction with log (cortisol) values is provided (Fig. 3).

## Discussion

### Main findings

This study points out the relevance of considering a combination of multiple stress-related factors: (i) cumulated life stressors (previous traumas, social support, and family functioning); and (ii) the biopsychological response to TPL diagnosis (salivary cortisol and  $\alpha$ -amylase as well as anxiety and depression symptoms) to predict the birth date in TPL women. According to previous research [38], the relationship between self-reported stress and biomarkers levels seems to be weak in TPL women. As for which stress measures are most strongly associated with preterm birth, lower family adaptation, higher BMI, and middle- and high-levels of cortisol in women with TPL diagnosis before 29 weeks of gestation

were the best predictors. Taking all these elements together, given multifactorial etiology of stress [37], and performing simultaneous analysis of both psychosocial (family adaptation) and biological variables (cortisol levels) as well as obstetric factors (BMI and the TPL diagnosis week) improves the birth date prediction better than when analyzing these factors separately [41].

## Strengths and limitations

The strengths of this study are as follows: (i) the inclusion of multiple stress-related variables; (ii) the successful follow-up of participants from TPL diagnosis to birth; (iii) the rigorous inclusion criteria to control additional stressful variables such as social exclusion or major medical illness; and (iv) the consideration of other potential obstetric predictors such as BMI, TPL diagnosis week, multiple pregnancy, *in-vitro* fertilization, or parity in the regression model. In turn, these strengths have limited generalizability of results and sample size. Furthermore, participants were not included if any data were missing or the follow-up was not completed, which could cause a selection bias. Finally, although all pregnant women at 24–31 + 6 weeks received similar treatment, individual differences in response to tocolytic agents and corticosteroids could have influenced biomarkers determinations.

## Clinical interpretation

Regarding stress biomarkers, middle- and high-cortisol levels in women with TPL diagnosis before 29 weeks of gestation predicted earlier birth date. In line with Campbell et al. (2005)[29] increasing levels of HPA axis biomarkers were relevant for determining birth date in TPL women. However, whereas they observed that stress biomarkers had a significant association with prematurity from the 28th week of gestation and onwards [29], our findings have shown this association from the 24th to 29th weeks of gestation. This discrepancy may be explained due to differences in follow-up length; whereas Campbell et al. (2005) [29] carried out a follow-up until 48 hours after TPL diagnosis, our research extended it until birth. With regard to  $\alpha$ -amylase levels, no differences have been observed, indicating that  $\alpha$ -amylase biomarker is not a significant variable to predict birth week in symptomatic women (see García-Blanco et al. (2017) for a similar finding) [28a, 28b]. In this case, previous research that found an association between  $\alpha$ -amylase levels and preterm birth suggested that this relationship may be restricted to women with prenatal depression [30].

As for psychological response to TPL, unlike previous studies that found an association between preterm birth and gestational anxiety [31, 32] and depressive symptoms [33–35], these self-reported symptoms after a TPL diagnosis have not been relevant to estimate the birth week in our study. Thus, this apparent inconsistency may be explained by inclusion of women without TPL diagnosis in previous studies. Undoubtedly, all women with a TPL diagnosis were expected to react with a subjective increase of anxiety and depressive symptoms. Nevertheless, only some of those women have shown middle- and high-cortisol levels. Therefore, this study has shown that stress biomarkers were stronger predictors than the subjective state anxiety for TPL women. Like it occurs with anxiety symptoms, suffering traumatic experiences previously to pregnancy may be associated with preterm birth in asymptomatic women [17, 19–20]. However, such differences have not been observed in this study with symptomatic women. It

could be expected that previous traumas are not relevant in this context because TPL represents a traumatic event itself. Hence, TPL can be considered as a pregnancy-specific traumatic event that may provoke a stress-vulnerability status in all cases [16].

Concerning chronic social stress-related factors, family adaptation was a relevant factor to estimate the birth week. Likewise, other studies with non-TPL women have concluded that some aspects of family functioning (e.g., poor emotional understanding by the partner) are related to preterm birth [21, 23]. In our study, family's ability to modify its rules, roles, and structure in response to environmental changes, rather than social support in general [22], was the most relevant social predictor [21, 23]. Therefore, among self-reported variables, chronic social stress has been a stronger predictor than psychological symptoms after a TPL diagnosis. Thus, TPL can trigger an increase of subjective anxiety but it cannot be a modulator of self-reported family functioning.

Finally, relating to obstetric variables, according to prior literature about preterm birth [52], actors such as maternal BMI and TPL week have also been relevant predictors for final birth date after a TPL in our study.

## **Conclusion**

This follow-up study uses a multidimensional approach to examine stress response in pregnant women with an antenatal adverse event such as TPL in order to explore potential indicators of vulnerability to preterm birth. These findings can be applied as a new useful tool to determine the birth date in TPL women by means of a simultaneous analysis of chronic social stressors (family adaptation) and the biological stress response to TPL diagnosis (salivary cortisol) together with obstetric conditions (BMI and TPL week). This study has important public health implications, since the number of preterm births may be reduced by initiating early preventive psychosocial interventions to increase family adaptation and decrease cortisol levels after a TPL as well as lose weight in symptomatic women.

## **Declarations**

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### **Conflict of interest**

The authors have no relevant financial or non-financial interests to disclose.

### **Ethics approval**

The Ethics Committee at the La Fe Health Research Institute approved the study protocol in 2015 (ref. 2015/0086) and informed consent was obtained from all participants.

### **Availability of data and material**

Collected data and materials of assessment comply with field standards and are available if required.

### **Author Contributions**

M. Vento: Protocol/project development; Funding acquisition; Supervision; Manuscript Writing/editing.

A. Moreno-Giménez: Data collection; Manuscript Writing/editing.

L. Campos-Berga: Data collection; Manuscript Writing/editing.

V. Diago: Protocol/project development; Funding acquisition; Supervision; Manuscript Writing/editing.

D. Hervás: Data analysis; Software; Manuscript Writing.

P. Sáenz: Protocol/project development; Data collection; Manuscript Writing/editing.

C. Cháfer-Pericás: Protocol/project development; Data analysis; Manuscript Writing/editing.

A. García-Blanco: Protocol/project development; Funding acquisition; Data collection; Supervision; Manuscript Writing/editing.

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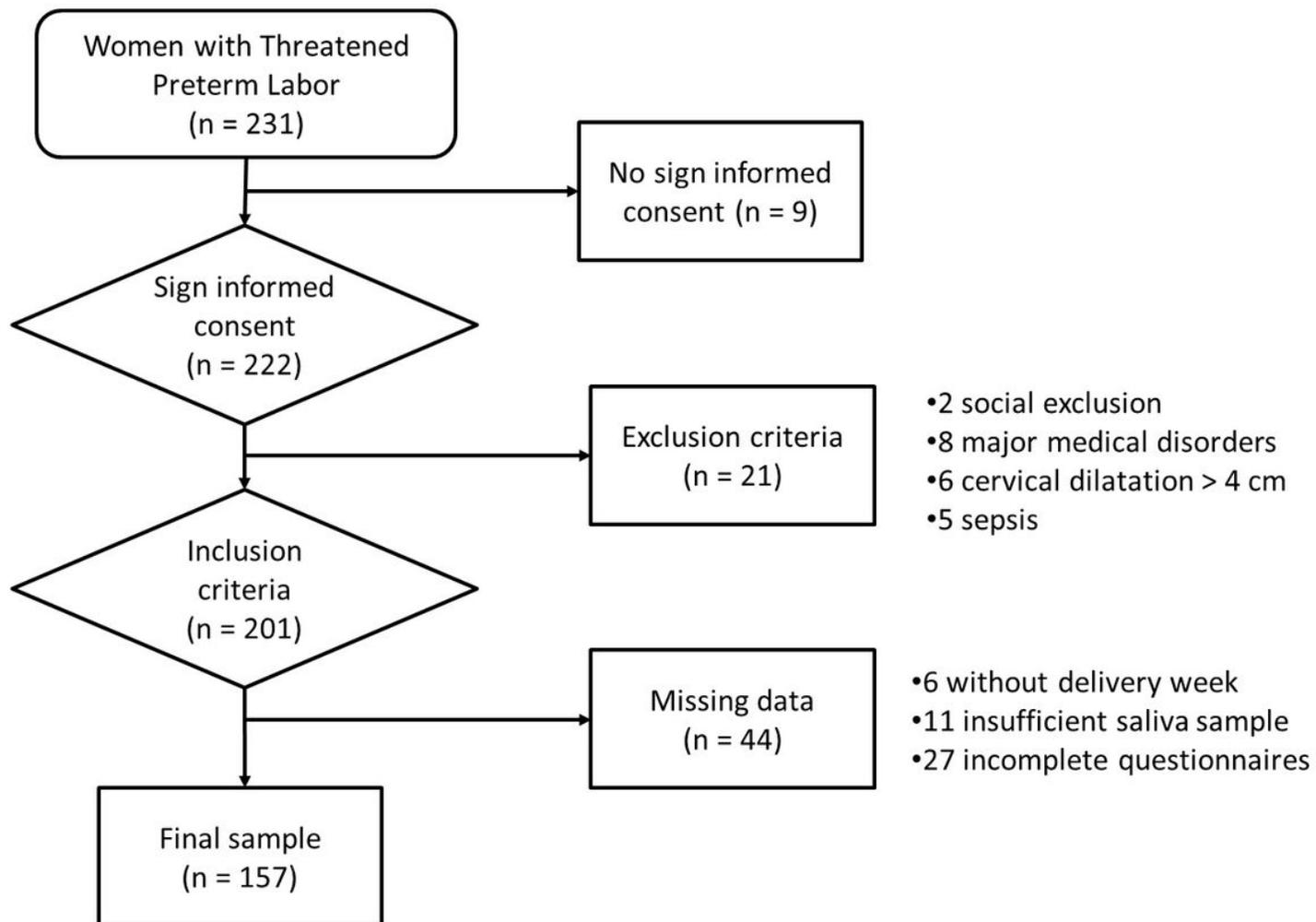
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## Figures



**Figure 1**

Flow diagram describing the recruitment process, the exclusion determinants, and the participants who completed the study.

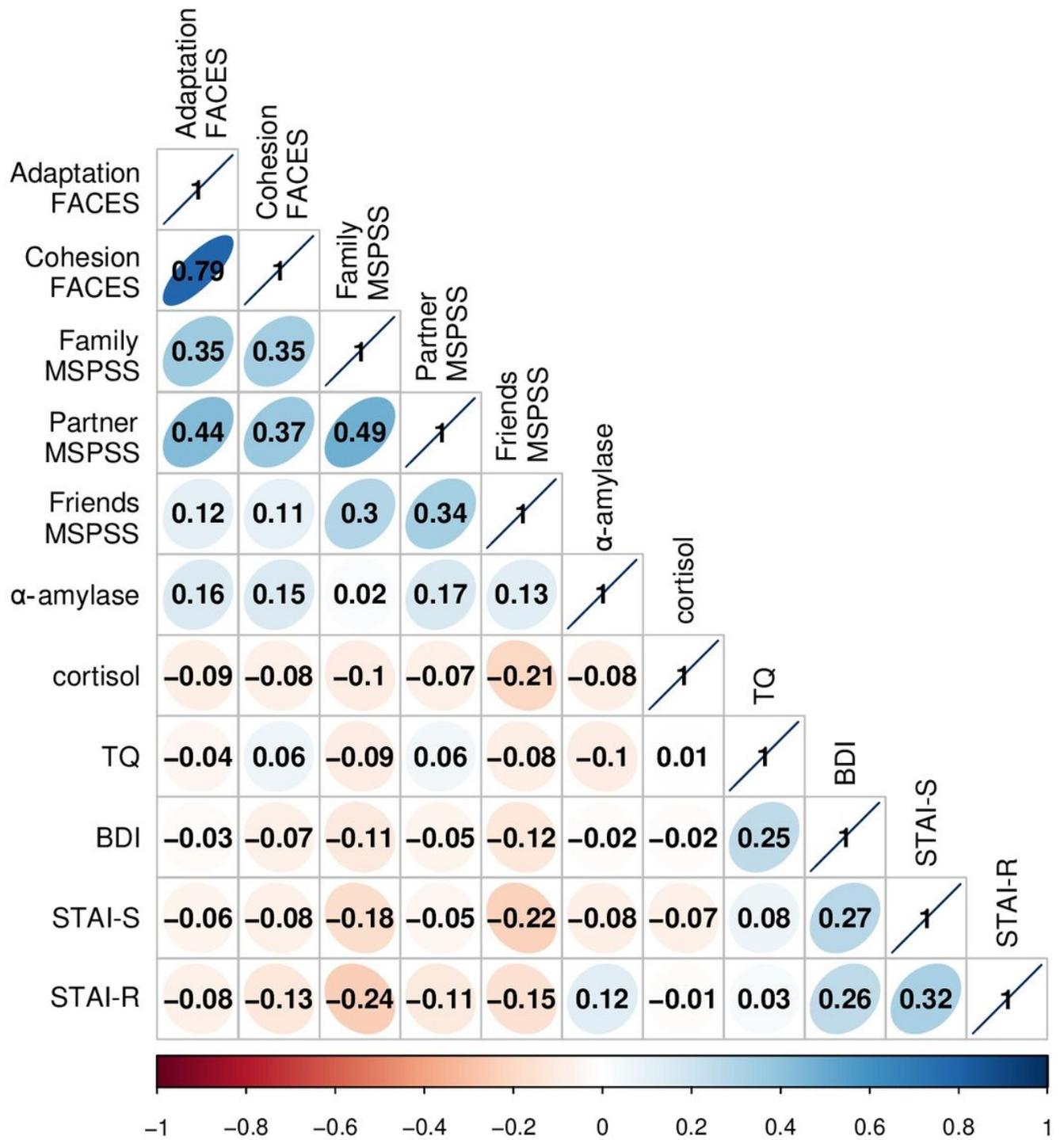
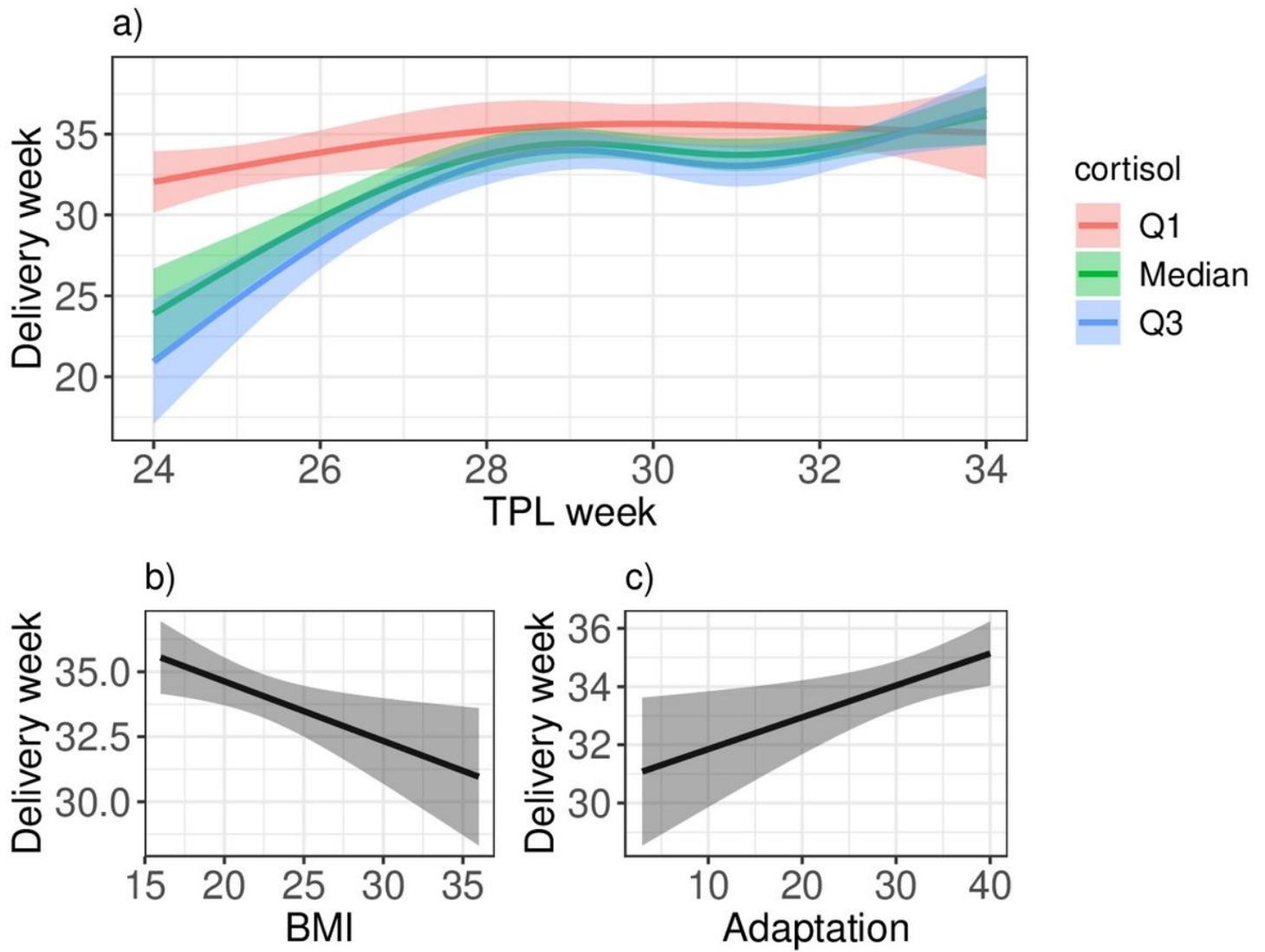


Figure 2

Correlation plot between the different stress-related variables.



**Figure 3**

Marginal effect plots depicting the relationship between labor week and a) log(cortisol) interaction with TPL week, b) Body Max Index, and c) family adaptation.