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FACULTY OF SCIENCES

Master of Statistics: Biostatistics

Masterproef

*Stress during pregnancy and its influence on the
offspring's development and temperament: a physiological
study*

Promotor :
dr. Nele GOEYVAERTS
Prof. dr. Cristina SOTTO

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Dr. BEA VAN DEN BERGH

José Elibiel Parra Soto

*Master Thesis nominated to obtain the degree of Master of Statistics , specialization
Biostatistics*

De transnationale Universiteit Limburg is een uniek samenwerkingsverband van twee universiteiten in twee landen:
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School of Social and Behavioral Sciences

Univariate and Joint Generalized Linear Mixed Models for the Analysis of Ordinal Data: One application to the Study of Depression and Anxiety During Pregnancy

Author: José E. Parra **Supervisors:** Prof Dr. Bea R.H Van den Bergh, Prof Dr. Goeyvaerts Nele.

Summary: Generalized Linear Mixed Models (GLMM's) are appropriate to model univariate and multivariate outcome data. These methods are especially suitable to analyze missing non-normal questionnaire data, accounting for longitudinal correlation and variability at subject level. Relationships between different outcomes can also be studied. GLMM's were used to study the associations between antenatal maternal anxiety (measured with The Pregnancy Related Anxiety Questionnaire PRAQ) and cortisol, as well as antenatal maternal depression (measured with the Edinburgh pregnancy depression scale EPDS) and cortisol. In total, there were 192 normal participants in the Prenatal Early Life Stress (PELS) PROJECT TILBURG. Univariate, bivariate and multivariate GLMM's for longitudinal correlated and missing data showed that the odds of having high levels of *fear about the baby's integrity* increases 1% per one unit of increase in cortisol in the first two trimesters and 7.2% in the last trimester (odds ratio of 1.01 p-value=0.0098). Higher levels of anxiety (PRAQ2) for higher cortisol levels of cortisol are expected, especially in the last period of pregnancy, nevertheless, this effect of cortisol varies according with the extent of fear in a particular pregnant woman. This result is consistent in the univariate and multivariate analysis. Finally, multivariate modeling shows that there is a strong relationship between all dimensions of PRAQ and the last dimension or *fear of changes in the mother's personal life and concern about future mother-child, father-child and partner relationship*.

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1. Introduction

Recent studies link high levels of psychological distress with poor birth and developmental outcomes [1]. For this reason the analysis of cortisol levels in pregnant women has been widely used, since this hormone is thought to be linked with effects of maternal psychological distress during pregnancy to fetal development [2].

In general, when individuals are confronted with stress, the hypothalamic pituitary-adrenal (HPA) axis initiates a complex set of neurophysiologic changes, including the synthesis and/or release of 3 key hormones — corticotrophin-releasing hormone, adrenocorticotrophic hormone, and cortisol [3]. The assessment of cortisol in saliva as a marker of HPA axis activity has become a standard procedure in psychosomatic medicine.

But measuring psychological distress in pregnant women in real life is complex and normally investigations on associations between psychological distress and cortisol during pregnancy are laboratory based studies using acute stressors. Few clinical studies have examined naturally occurring stressors and in any case most of the studies have reported non-significant or low associations. This is especially true when using single measures of cortisol from amniotic fluid, serum or saliva, but even in cases where the diurnal variation in cortisol is measured, associations between psychological distress and cortisol are not clear [2].

Methodological differences between these studies can explain some of the variations in findings, including for example single versus multiple days to become an improved estimation of the variation in cortisol, as well as incorrect variable inclusions and poor statistical analysis.

Measuring psychological distress usually involves questionnaires that create univariate and multivariate non normal distributed variables, characterized by missing values and skewed distributions. This type of data comes as a rule in the form of counts, scores and ordinal or nominal scales that make the statistical analysis complex and determinant.

Other type of limitations of these studies comes from the fact that they focus on overall differences between average levels of psychological distress and cortisol. The types of questions that drive these studies are to determine whether high levels of psychological distress in pregnancy exhibit high levels of cortisol. Answering these types of questions result in findings at population levels which happen to be uninformative about the process within individuals.

Nowadays there are appropriate methods to satisfactorily answer researching questions at population and patient level while coping with missing data.

1.1 Current Study

This study was designed to examine the association between psychological distress and cortisol during pregnancy in 192 patients. More specific, the objective of this study was to determine whether trimester-to-trimester changes in anxiety (measured using PRAQ questionnaire) and depression (using EPDS questionnaires) in pregnant women were affected by trimestrial changes in Cortisol while controlling for baseline factors like Immediate Anxiety (measured by the STATE and TRAIT questionnaire), age, having lost a child, smoking status and having had children in the past.

2. Data Description

2.1 Participants

Data of the group of 192 pregnant women that are used in this analysis come from the Prenatal Early Life Stress (PELS) PROJECT TILBURG, collected during May 2008 and September 2011 in the School of Social and Behavioral Sciences in Tilburg University. There was not exclusion criteria. Descriptive information for the study sample is shown in Table 1.

Table 1. Descriptive statistics of the 192 participants in the PELS Project

Descriptive Statistics				
	Missing			Missing
Age	7.8%	Marital Status		
Min Age	18	Living-Partner	42	0.52%
Max Age	43	Living-Together	38	
Mean	32	Married	97	
		Other	1	
		Registered-Partners	9	
Education Level	0.52%	Single	4	
Basic	1			
Higher Vocational	74	Lost a child		0.26%
LBO/MAVO	18	No	134	
Posgraduate	7	Yes	53	
University	42			
Vocational	49	Children		0.52%
		No	89	
Smoke	0.2%	Yes	102	
Not	141			
Yes Daily	39 0%			

Yes Weekly	4		
Yes Less than once per week	4	Total Sample Size	192

2.2 Questionnaires

2.2.1 Edinburgh pregnancy depression scale

The Edinburgh pregnancy depression scale (EPDS) is a short 10-item questionnaire that is commonly used in the postnatal period to look for postnatal depression [4] but it is also validated for use during the prenatal period in order to assess maternal depression [5]. The EPDS is the most appropriate questionnaire to use in the perinatal period as it eliminates somatic symptoms of depression, which are common in pregnancy and the postpartum and not necessarily indicative of psychological problems (Cox et al., 1987). Milgrom et al (2005) showed that for a cut-off score of 13 or more there is an 85% sensitivity and 71% specificity identifying major and minor depression in the postnatal period (Milgrom et al., 2005).[6]

Score 0-9 When scores are in this range this may indicate the presence of some symptoms of distress that may be short-lived and are not likely to interfere with day to day ability to function at home or at work. However if these symptoms have persisted more than a week or two further enquiry is warranted as to the cause

Score 10-12 Scores within this range indicate the presence of symptoms of distress that may be discomforting. The EDS should be repeated within 1-2 weeks and if the scores increase to above 12 referral to a mental health specialist or general practitioner should be considered for review.

Score 13 and higher Scores above 12 require further evaluation and possible referral to a perinatal mental health specialist. The EDS should be repeated regularly to monitor progress.

2.2.2 Pregnancy Related Anxiety Questionnaire

The Pregnancy Related Anxiety Questionnaire (PRAQ) was based on a questionnaire constructed by Taylor [7] and on information from other articles [16] [22] [23], and it is composed by five different indicators of anxiety in pregnant women called subscales. These subscales are *concern for oneself and the partner relationship during pregnancy* (PRAQ1), *fear of the integrity of the baby* (PRAQ2), *fear of delivery* (PRAQ3), *fear of changes in the mother's personal life* (PRAQ4) and *concern about future mother-child, father-child and partner relationship* (PRAQ5) [8].

2.2.3 The Spielberger State-Trait Anxiety Inventory

The *State-Trait Anxiety Inventory* (STAI) is a commonly used measure of trait and state anxiety [24]. It can be used in clinical settings to diagnose anxiety and to distinguish it from depressive syndromes. It has 20 items for assessing trait anxiety and 20 for state anxiety. State anxiety items include: “I am tense; I am worried” and “I feel calm; I feel secure”. Trait anxiety items include: “I worry too much over something that really doesn’t matter” and “I am content; I

am a steady person.” All items are rated on a 4-point scale (e.g., from “Almost Never” to “Almost Always”). Higher scores indicate greater anxiety.

Internal consistency coefficients for the scale have ranged from .86 to .95; test-retest reliability coefficients have ranged from .65 to .75 over a 2-month interval [24]. Test-retest coefficients for this measure in the present study ranged from .69 to .89.

Only STATE anxiety was measured in the three trimesters in contrast with TRAIT anxiety which was only measured in the last two trimesters. For this reason TRAIT anxiety is not considered. This is completely feasible since STATE and TRAIT are completely distinguishable and measure different tendencies toward anxiety. TRAIT anxiety refers to being anxiety-prone, or in other words, it is a stable personality characteristic. In contrast, STATE anxiety refers to the immediate feelings of being anxious, such as nervousness and bodily tension. STATE anxiety often has a more direct influence on thinking and behavior than does TRAIT. High state anxiety may interfere with concentration and performance of tasks [21]. For this reason, it was of interest to use STATE anxiety as a baseline variable when measuring anxiety in pregnant women with the PRAQ questionnaire.

2.3 Psychological Measurements: Cortisol

During pregnancy the mother collected cortisol herself using salivates at home. Each participant had to take 8 samples during each pregnancy trimester. The samples per trimester were divided over two consecutive days, four samples each day. They had to take the first samples when they were still lying in their bed just after waking up, the second sample 30 minutes later (without eating or brushing their teeth), the third sample 6 hours later and the fourth sample 12 hours after they woke up.

The collected samples were stored at -20°C and shipped to the Kirschbaum Institute (Dresden University of Technology, Germany) for analysis. After thawing, saliva samples were centrifuged at 3000 rpm for 5 min. Salivary free cortisol was analyzed using chemiluminescence immunoassay (CLIA; IBL Hamburg, Germany). The precision of the intra- and inter-assay variability for the used technique is less than 10%. The lower detection limit of this assay is .43 nmol for a 50 µl salivary sample. All samples of one subject were analyzed in the same run to reduce error variance caused by imprecision of the assay.

2.4 Exploratory Data Analysis

2.4.1 The EPDS Questionnaire

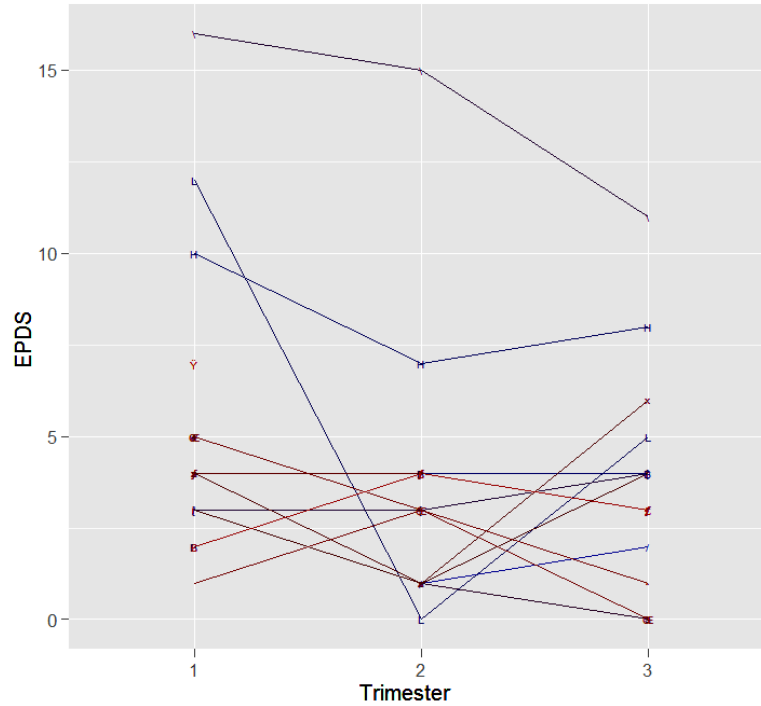
Depression was measured using the EPDS questionnaire. Data results of the measurement were composed by counts that ranged from 0-21 with a median of 4 and a mean of 5.097 and with a standard deviation of 4.3. In total EPDS had 73 missing values among 192 patients measured three times, which corresponds to a missing percentage of 13%. The following Table shows the descriptive statistics of chronic mood per trimester.

Table 3. Summary Statistics of Depression from a total of 192 patients

Descriptive statistics of EPDS by gestation trimester											
First Trimester				Second Trimester				Third Trimester			
Mean	S.D	Range	Mis.	Mean	S.D	Range	Mis.	Mean	S.D	Range	Mis.
5.62	4.19	0-17	15(8%)	4.30	3.96	0-20	21(11%)	5.39	4.59	0-21	38(20%)

Table 3 shows how the number of missing data increases with time. The missing data increased from 7.8% to 20% in the Third Trimester. High variability is also present in the EPDS scores per trimester, despite the fact that Table 3 shows marginal standard deviations, it can be seen in Figure 1 that intra subject and between subjects variability are very high. Finally, the distribution of the EPDS distribution of the scores is highly skewed to the left indicating a possible Poisson distributed variable (Figure 1A in the appendix).

Figure 1. EPDS profiles of 20 randomly selected patients.



2.4.2 The PRAQ Questionnaire

The PRAQ questionnaire is composed by 20 items (affirmations) which have to be answered by indicating how “applicable” this affirmation is related with the current situation of the patient. In total there are up to seven levels of the response. Level one for example is “absolutely not applicable”, level fourth indicates “Middle applicable” and level seven corresponds to “Very applicable”. These 20 items express different areas or subscales of anxiety, namely , “*Concern for oneself and the partner relationship during pregnancy*”, “*fear of the integrity of the baby*”, “*fear of delivery*”, “*fear of changes in the mother's personal life*” and “*Concern about future mother-child, father-child and partner relationship*”. Summary statistics of PRAQ’s five anxiety subscales in the participants are shown in Table 4.

For simplicity these subscales will be presented as “*Relationship*”, “*Child’s Integrity*”, “*Fear Delivery*”, “*Mother’s Life*”, “*Relationship Child*” respectively. Then we have one outcome variable (anxiety) composed by 5 outcomes measured each trimester. In average there were 24 missing values in each of the five PRAQ variables.

Table 4. Descriptive statistics PRAQ per pregnancy trimester

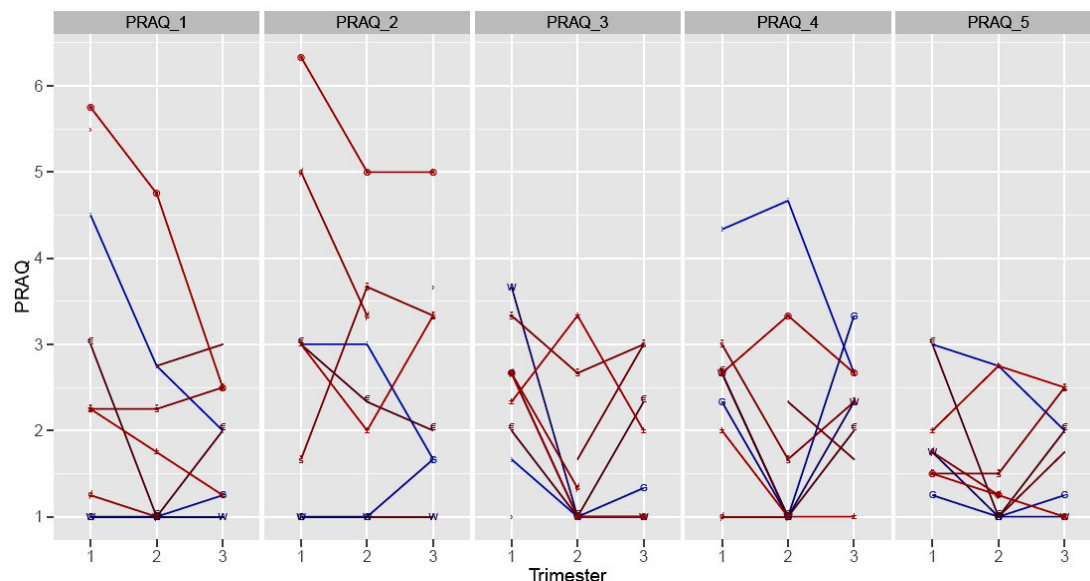
PRAQ	Dimensions Pregnancy Related Anxiety Questionnaire									
	Relationship			Child’s Integrity			Fear Delivery			
Pregnancy Period	Range	Mean	Std	Range	Mean	Std	Range	Mean	Std	Missing
1	1-6.5	2.9	1.45	1-6.7	2.6	1.43	1-7.0	2.5	1.4	14(6.2%)
2	1-7.0	2.7	1.5	1-7.0	2.6	1.4	1.6.3	2.4	1.5	21(11%)

	3	1-7.0	2.6	1.4	1-7.0	2.8	1.4	1-6.0	2.3	1.3	37(19%)
		Mother's Life			Relationship Child						
Pregnancy Period		Range	Mean	Std	Range	Mean	Std	Missing			
1	1-6.7	2.6	1.4	1-4.5	2	0.97	14(7%)				
2	1-6.0	2.2	1.3	1-5.5	1.9	0.97	21(11%)				
3	1-6.0	2.2	1.3	1-5.2	1.9	0.92	37(19%)				

Notice there seems to be a reduction in the mean of PRAQ throughout the pregnancy periods for almost all of the dimensions. It is also interesting to see how in the last period of pregnancy the mother's fear of the Integrity of the child seems to increase .

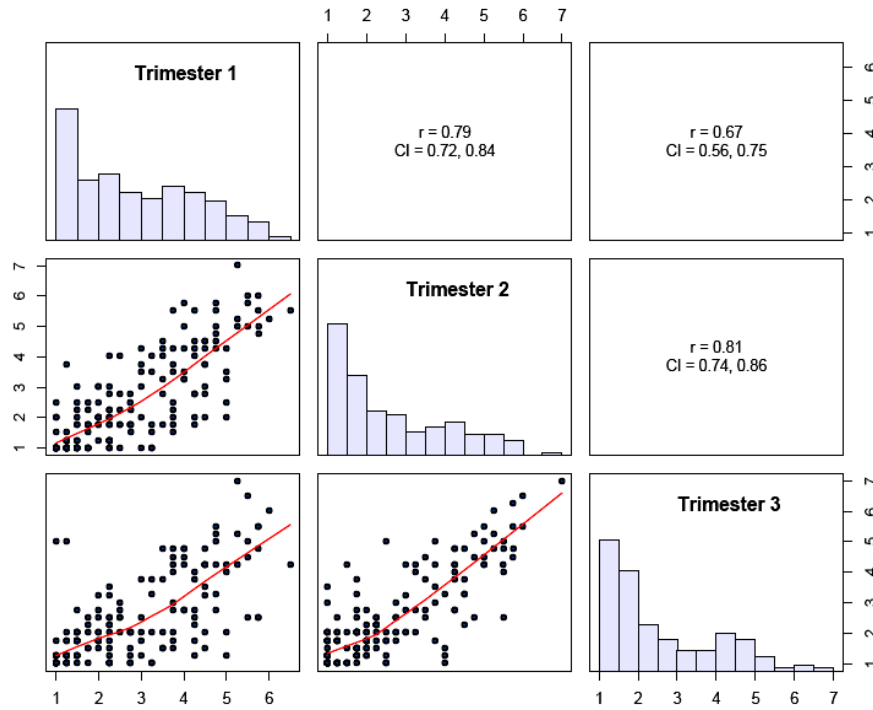
In any case these means are the pooling of the information of all patients with available measures in particular trimesters, therefore these means can be biased due to the fact that only complete cases are averaged and because of the variability within patient which is very high. Figure 5 shows in this respect, the profiles of 10 randomly selected participants. Notice the big contrast of the means in Table 4 with the profiles in Figure 5. This suggests that an analysis that takes into account patient variability is more advantageous than a population level analysis.

Figure 5. Random (10) profiles of PRAQ per trimester.



With the aim of understanding better the nature of the PRAQ dimensions, correlations were calculated between and within dimensions. Figure 2 shows only the distribution of the first dimension, but the other dimensions are included in the appendix. As expected, correlations of PRAQ scores are highly correlated per trimester. Figure 2 shows the distribution of “*Concern about oneself and partner relationship*” (PRAQ1). A spearman correlation test with a 95% confidence interval is added

Figure 2. Distribution of first dimension of PRAQ Concern about oneself and partner relationship and its Spearman correlations (with confidence intervals) per trimesters.



Despite the fact that PRAQ dimensions are considered to be ordinal in nature, Figure 2 shows that not all levels are integers, the reason of this discrepancy is that these are the scores resulting from the averaging of the 20 question items of PRAQ into each of its subscales. In any case, to keep PRAQ dimensions ordinal in nature, further analysis was made grouping scores to the closest PRAQ level.

In general, correlations between PRAQ dimensions are not high (Figures 2A, 3A, 4A and 5A in the appendix), it is noticing that PRAQ5 or “Concern about future mother-child, father-child and partner relationship”, has the highest correlated with other dimensions. Variances per trimester tend to be constant and it ranges of variances where 2.1-2.09, 1.95-2.1, 1.74-1.98, 1.60-1.93 and 0.85-0.95 for the PRAQ dimensions “Relationship”, “Child’s Integrity”, “Fear Delivery”, “Mother’s Life”, “Relationship Child” respectively. This indicates that the assumption of constant variance of these scores throughout the time could be reasonable (as shown in Table 4). Finally, the other fourth anxiety dimensions of PRAQ show also skewed distributions (Figures 2A, 3A, 4A and 5A in the appendix) and similar comments over the variance structure can be drawn.

2.4.3 A physiological measure: cortisol

Cortisol was measured four times per day during two consecutive days in three occasions (Trimester 1, Trimester 2 and Trimester 3). This makes eight measures per two days in a particular trimester. In order to have a more precise and comparable lecture of cortisol, these four daily measurements were averaged according to the hour of the measurement. In case of just having one out of two measurements, the available measurement was kept as the average for that particular hour.

In figure 3 the variation of these means of cortisol can be appreciated measured in different hours during the day. Lowest values appear to be at the end of the day before sleeping. Nevertheless it can be seen in figure 3 that the behavior of cortisol presents high variation between and within participants. It is also worth noticing how the hormone scores tend to have a patterns with mode in the second measure of cortisol during the day (measure of cortisol after 30 minutes of awakening).

Figure 3. Individual Cortisol profiles per day and Trimester. Cortisol Sample axis represents each one of the fourth measures of cortisol taken during the day in the first, second and third trimester respectively.

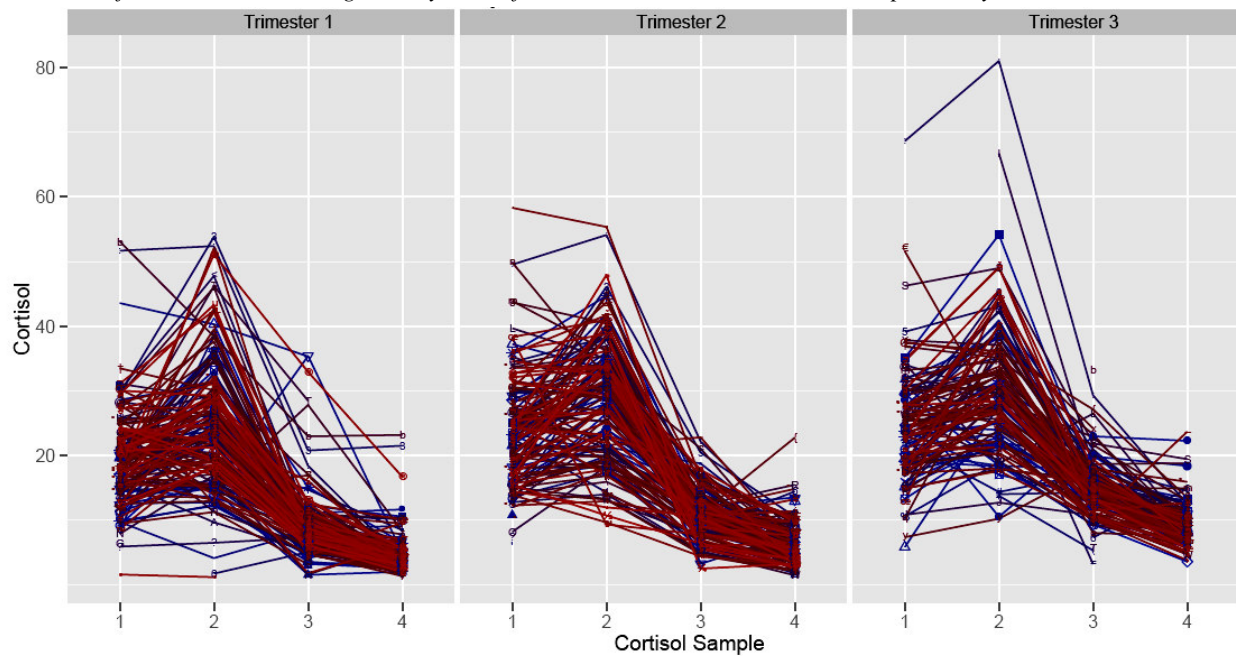


Figure 3 shows the profiles of cortisol during the day labeled by *Cortisol Sample*. Cortisol sample of value 1 indicates that the measurement of cortisol was the first in the day; Cortisol Sample of value 2 indicates that the measurement of cortisol was the second and so on. Cortisol measurements are also divided by trimester, then trimester one indicates the measures of cortisol taken in *Trimester 1* and so on. Each line represents one subject. Summary statistics of cortisol

measurements per trimester and within trimester of cortisol in Tilburg Study are given in Table 5.

Table 5. Summary Statistics of daily Cortisol measurements (four measures in one day) per trimester

Trimester	First Cortisol Measurement Summary				
	Min	Mean	Std	Max	Missing
1	1.622	19.48	7.35	53.11	39
2	6.406	23.83	8.66	58.32	50
3	5.852	24.67	8.40	68.63	69
Second Cortisol Measurement					
1	1.207	24.83	9.88	53.84	38
2	9.347	28.52	9.20	55.36	47
3	10.25	30.09	10.34	81.03	67
Third Cortisol Measurement					
1	1.568	9.11	5.33	35.42	29
2	2.532	10.92	3.73	22.83	44
3	3.221	14.9	4.47	33.3	67
Fourth Cortisol Measurement					
1	1.276	4.771	2.99	23.2	33
2	1.494	6.072	3.18	22.78	42
3	3.606	9.433	3.39	23.71	60

Notice how in general, the means of cortisol in Table 5 tend to increase with time of pregnancy in contrast with the reduction of PRAQ means in Table 4.

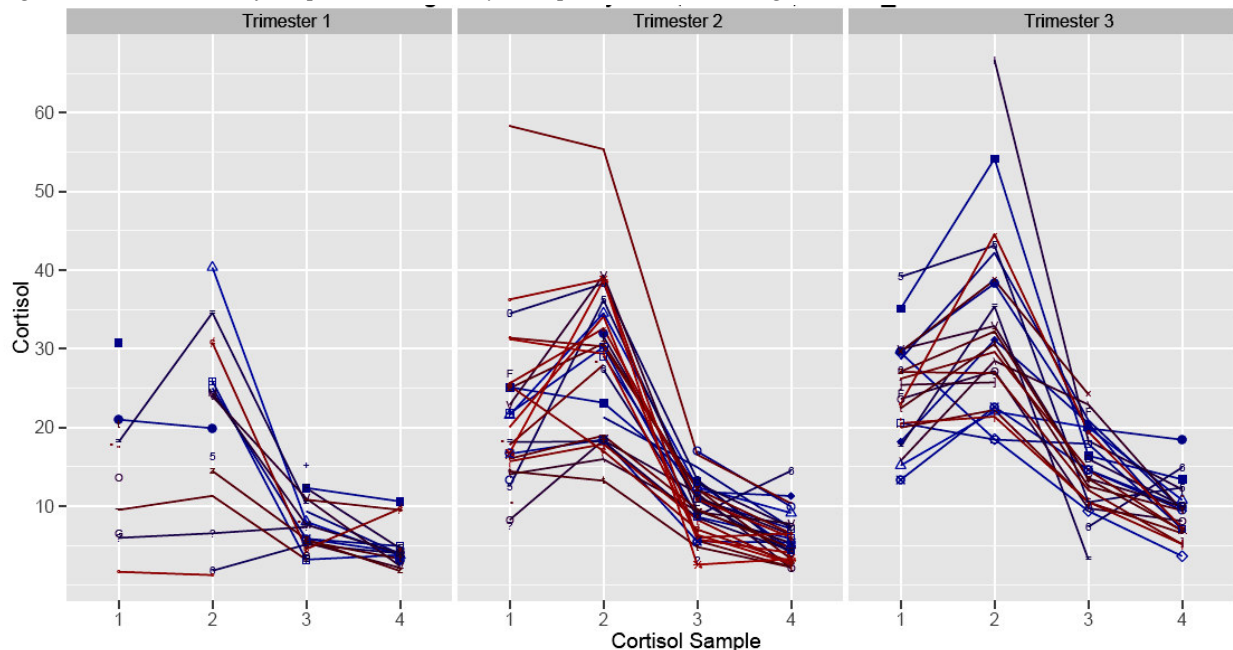
2.4.3.1 Summary Indicators of Repeated Cortisol Measurements

It is of interest to study the association between psychological distress and cortisol during pregnancy, but in particular, to study if there is an effect of this hormone in depression and anxiety among pregnant women. Cortisol was measured four times per day per trimester and depression and anxiety only once. For this reason cortisol had to be summarized in order to use it as a covariate in the analysis.

There are different ways of summarizing these psychological measurements, for instance by measuring the *area under the curve*, *Cortisol Awakening Response CAR* and *slopes of linear regressions per patient* [27]. None of these methods are indicated here because of missing values. Figure 4 gives an idea of the profiles of cortisol in individuals with one or more missing values. Patterns of missing values differ. For instance, there were 23 patients without a single value of cortisol and 17 patients with only one measure of cortisol in the first trimester.

It can be observed in Figure 4, that CAR is not a good summary statistic to describe levels of cortisol per trimester, since the first and/or the second measurement of cortisol in several cases is/are not available. Then making *a simple average* of the cortisol scores, or making *short cortisol diurnal profile* (only mean of scores 1, 3, 4), or using the *evening cortisol sample* (sample 4) was recommended.

Figure 4. Individual Profiles per trimester only with patients with missing cortisol data



Another disadvantage of using CAR can be the potential negative values that this summary of cortisol can arise, since the second *Cortisol Sample* can be lower in value than the first one.

Recent studies have indicated significantly altered awakening rises in various conditions related to psychopathology. It is positively associated with perceived chronic stress [25] [33] [34] [35], neuroticism [36] as well as with depressive symptomatology [33]. Participants in the Prenatal Early Life Stress (PELS) project were healthy in general. The distribution of the participants that ever had a disease or were treated because of physical or mental difficulties was as follows: "Anxiety" (2% patients treated) , "Cardiovascular Diseases"(2%), "Depression"(8%), "Depression Now" (0.5%) "Lung Disease"(1%), "Always Healthy"(66%) , "Treated Lungs" (4%) , "Treated Others"(5%) , "Treated Others Now"(8%). Negative CAR then could be related with physical or mental morbidity among the group or, negative CAR scores can have relation with the compliance of participants, since in spite of dramatic alteration of cortisol levels (maternal cortisol increases 2-4-fold over the course of pregnancy), the circadian rhythm (with a peak shortly after awaking and gradually declining over the day) remains intact during pregnancy [2][26].

Then, it is possible that these subjects showing a negative response really do have a deviant response to awakening. However, an alternative explanation might be that subjects woke up earlier than they reported, and that their data therefore represent the down stroke of the morning acrophase.

Similar studies indicate that waking up earlier than reported can be responsible for the majority of the apparent negative cortisol awakening responses [37]. In any case, precautions about compliance were not considered in the study, making hard to clarify the extent of non compliance, but it was found that among the participants with at least one available measure of CAR (188 in total), the percentage of participants with at least one negative value of CAR was of 60% (113 participants in total). It was also found that 18% of the participants (20 among 113) had higher negative CAR in late reported hours after awakening, but not important relations about time and hour of awakening were visible (see Figure 1C in the appendix). Table 2 shows the summary statistics of the group of patients from which the CAR was negative. Notice that means and medians are far away from being zero.

Table 2. Frequencies of negative Cortisol Awakening response.

Summary Negative CAR among participants							
	Number Patients	Min.	Max.	Median	Mean	S.D	Missing
CAR Trimester 1	38(20%)	-17.05	-0.1228	-3.414	-4.373	3.99	47(24%)
CAR Trimester 2	43(22%)	-28.82	-0.2063	-2.5	-4.024	5.08	57(30%)
CAR Trimester 3	25(13%)	-25.7	-0.1625	-2.798	-5.469	6.34	75(39%)

Summarizing what was said: negative CAR scores can make sense from a particular morbidity state, but also can indicate possible non compliance. Negative CAR scores, combined with the impossibility of calculating CAR scores for 21% of the patients indicate that other methods of summarizing cortisol should be considered.

3. Methodology

Distribution characteristics of the PRAQ and EPDS outcomes require using Generalized Linear Mixed Models and taking into account the missing data.

3.1 Missing data treatment

In a longitudinal psychological study, each subject is measured repeatedly on the same outcome at a number of time points. In general, the results come from the scoring of questionnaires which are characterized by non-normality and missing data.

In the presence of missing data or wrong model assumptions, the standard methods of longitudinal analysis may yield biased estimates and standard errors. Thereby the choice of analytical method has important implications on the estimates of outcome variables and the relationship between outcome and covariates. Thus the mechanisms producing the missing observations as well as the model assumptions should be considered.

In the present study, different methods of missing data treatment were used: Direct Likelihood where the data is used as it is, and multiple imputation. Using the terminology described by Little and Rubin [9], missing data mechanisms can be classified as *missing completely at random (MCAR)* if the probability of missing information at each occasion is independent of both the observed and the missing values of the response, *missing at random (MAR)* if the probability of dropout at each occasion is related to the observed responses, but not the missing values and *missing not at random (MNAR)* if the probability of missing numbers depends on unobserved responses.

When the data are MCAR marginal analysis using Generalized Estimating equations for instance, gives consistent estimates for the parameters. While under MAR or non-ignorable assumptions, estimates are not unbiased. Different methods have been developed to handle missing data under different missing mechanisms. Multiple Imputation and Direct Likelihood have been developed to deal with biases that may result from incomplete data which are MAR

3.1.1 Multiple Imputation

When MCAR or MAR holds, the missing data are ignorable when likelihood-based methods are used like Generalized Estimating Equations (GEE) and Generalized Linear Mixed Model (GLMM) [9][10]. The disadvantage of using GEE is that the missing mechanism is assumed to be MCAR (unless an imputed data is used) and in general this is not the case, especially in questionnaire data where the participants avoid answering questions that they for example consider private or, in large questionnaires, missing values are the result of the indifference or lack of time of the participants. Then in the context of MAR, multiple imputations in the responses and covariates are relevant.

Multiple Imputation was formally introduced by Rubin in 1978. The key idea was to impute each missing value with a set of M plausible values. These M plausible values represent the uncertainty about the right value to impute.

After the imputation step, the imputed datasets are then analyzed separately using procedures for complete data and then the results are combined in a single analysis.

3.1.1.1 The imputation Step

The first step in the imputation process is to define the way of imputing. There are different methods for imputing M values in a dataset. We consider the “Chained Equations Method”. The idea is to specify a imputation model separately for each variable.

Let the hypothetically complete data Y be a partially observed random sample from the p -variate multivariate distribution $P(Y | \theta)$. We assume that the multivariate distribution of Y is completely specified by θ , a vector of unknown parameters. The problem is how to get the multivariate distribution of θ , either explicitly or implicitly. The idea is to obtain this posterior distribution by sampling iteratively from conditional distributions of the form

$$\begin{aligned} &P(Y_1 | Y_{-1}, \theta_1) \\ &\dots \\ &P(Y_p | Y_{-p}, \theta_p) \end{aligned}$$

The parameters $\theta_1, \dots, \theta_p$ are specific to the respective conditional densities and are not necessarily the product of a factorization of the ‘true’ joint distribution $P(Y | \theta)$. Starting from a simple draw from observed marginal distributions, the t -th iteration of chained equations is a Gibbs sampler that successively draws

$$\begin{aligned}
\theta_1^{*(t)} &\sim P(\theta_1 | Y_1^{obs}, Y_2^{(t-1)}, \dots, Y_p^{(t-1)}) \\
Y_1^{*(t)} &\sim P(Y_1 | Y_1^{obs}, Y_2^{(t-1)}, \dots, Y_p^{(t-1)}, \theta_1^{*(t)}) \\
&\dots \\
\theta_p^{*(t)} &\sim P(\theta_p | Y_p^{obs}, Y_1^{(t)}, \dots, Y_{p-1}^{(t)}) \\
Y_p^{*(t)} &\sim P(Y_p | Y_p^{obs}, Y_1^{(t)}, \dots, Y_p^{(t)}, \theta_p^{*(t)})
\end{aligned}$$

where $Y_j^{(t)} = (Y_j^{obs}, Y_j^{*(t)})$ is the j-th imputed variable at iteration t. Observe that previous imputations $Y_j^{*(t-1)}$ only enter $Y_j^{*(t)}$ through its relation with other variables, and not directly. Convergence can therefore be quite fast, unlike many other MCMC methods [17].

3.1.1.2 Pooling The Information

After obtaining the M analysis the information of the estimated parameters is combined as follows:

When data would be complete, suppose that inference about the parameter β is made by $(\beta - \hat{\beta}) \sim N(0, U)$. The M within-imputation estimates for β are pooled to give the multiple imputation estimate $\hat{\beta}^* = \frac{\sum_{m=1}^M \hat{\beta}^m}{M}$. Furthermore, one can make normal-based inferences for β

based upon $(\beta - \hat{\beta}^*) \sim N(0, V)$, where $V = W + \left(\frac{M+1}{M}\right)B$. $W = \frac{\sum_{m=1}^M U^m}{M}$ is the average within

imputation variance, and $B = \frac{\sum_{m=1}^M (\hat{\beta}^m - \hat{\beta}^*)(\hat{\beta}^m - \hat{\beta}^*)^T}{M-1}$ is the between imputation variance [9][10].

3.2 Univariate Generalized Linear Mixed Models (GLMM)

For analysis of longitudinal data, random subject effects can be added into the regression model to account for the correlation of the data. The resulting model is a mixed model including the usual fixed effects for the regressors plus the random effects.

For count and ordinal data the generalized linear mixed models (GLMMs) have been developed, these methods extend GLMs by the inclusion of random effects in the predictor.

Using the notation of Molenberghs [11], consider y_{ij} as the j th outcome measured for cluster or subject i , $i = 1, 2, \dots, N$, $j = 1, 2, \dots, n_i$. y_i denotes the vector of all measurements for the i -th subject, that is, $y_i = (y_{i1}, \dots, y_{in_i})$. It is assumed that conditionally on q -dimensional random effects b_i are drawn independently from a Normal Distribution $N(0, D)$. The outcomes y_{ij} have densities of the form:

$$f_i(y_{ij} | b_i, \beta, \phi) = \exp\{\phi^{-1}[y_{ij}\theta_{ij} - \psi(\theta_{ij})] + c_{ij}(y_{ij}, \phi)\}$$

With $g(\mu_{ij}) = \eta_{ij}(E(y_{ij} | b_i, x_{ij}) = x'_{ij}\beta + z'_{ij}b_i$ for a known link function $g(\cdot)$, with x_{ij} and z_{ij} k -dimensional and q -dimensional vectors of known covariate values, with β a k -dimensional vector of unknown fixed regression coefficients and with ϕ a scale parameter. Finally, let $f_i(b_i | D)$ denote the density of the $N(0, D)$ density distribution for the random effects b_i .

Including the random effects, the expected value of the outcome variable, which is related to the linear predictor via the link function is given as $\mu_{ij} = E(y_{ij} | b_i, x_{ij})$. This is the expectation of the conditional distribution of the outcome given the random effects. As a result, GLMM are often referred to as conditional models.

3.2.1 Poisson GLMM

For count data, various types of Poisson mixed models have been proposed. Let Y_{ij} be the value of the count variable (where Y_{ij} can take values 0,1,2,...) associated with individual i and time point j . If this count is assumed to be drawn from a Poisson distribution, then the mixed Poisson regression model indicates the expected number of counts as $\log \mu_{ij} = \eta_{ij}$, with the linear predictor $\eta_{ij} = x'_{ij}\beta + z'_{ij}b_i$. Assuming the Poisson process for the count Y_{ij} , the probability that $Y_{ij} = y$, conditional on the random effects is given as $P(Y_{ij} = y | b_i, x_{ij}) = \exp(-\mu_{ij}) \frac{(\mu_{ij})^y}{y!}$

3.2.2 GLMM For Ordinal Outcomes

Normally, the proportional odds model described by McCullagh [29], which is based on the logistic regression formulation, is a common choice for analysis of ordinal data. The proportional odds model expresses the ordinal responses in C categories ($c = 1, 2, \dots, C$) in terms of $C - 1$ cumulative category comparisons, specifically, $C - 1$ cumulative logits (i.e., log odds). Here, denote the conditional cumulative probabilities for the C categories of the outcome Y_{ij} as

$P_{ijc} = P(Y_{ij} \leq c | b_i, x_{ij}) = \sum_{c=1}^C p_{ijc}$ where p_{ijc} represents the conditional probability of response in category c . The logistic GLMM for the conditional cumulative probabilities is given in terms of

the cumulative logits as $\log \text{it}(P(Y_{ij} \leq c | b_i)) = \log \left[\frac{\mu_{ijc}}{1 - \mu_{ijc}} \right] = \eta_{ijc}$ ($c = 1, 2, \dots, C - 1$) [9], where the

linear predictor is $\mu_{ijc} = \gamma_c - [x'_{ij}\beta + z'_{ij}b_i]$, with $C-1$ strictly increasing model thresholds γ_c (i.e., $\gamma_1 < \gamma_2 \dots < \gamma_{C-1}$). The thresholds allow the cumulative response probabilities to differ. For identification, either the first threshold γ_1 or the model intercept β_0 is set to zero as the regression coefficient does not carry c subscript, the effects of the regressors do not vary across categories. This assumption is commonly called the identical odds ratios across the $C - 1$ cutoffs the proportional odds assumption.

Because the ordinal model is defined in terms of the cumulative probabilities, the conditional probability of a response in category c is obtained as the difference of two conditional cumulative probabilities: $p(Y_{ij} = c | b_i, x_{ij}) = \Psi(\eta_{ijc}) - \Psi(\eta_{ijc-1})$. Here, $\gamma_0 = -\infty$ and $\gamma_c = \infty$, and so $\Psi(\eta_{ij0}) = 0$ and $\Psi(\eta_{ijC}) = 1$

3.3 Generalized Linear Mixed Models for joint modeling

The joint model assumes a mixed model for each outcome, and these univariate models are combined through specification of a joint multivariate distribution for all random effects. The joint model can be considered as a new mixed model with the form $y_i | b_i \sim F_i(\psi, b_i)$, that is, conditional on a random effects vector of a higher dimension. y_i follows a prespecified distribution F_i , possibly depending on covariates and parameterized through ψ . Let Θ^* be the vector containing all fixed effects and covariance parameters, then $l_i(Y_{1i}, Y_{2i}, \dots, Y_{mi} | \Theta^*)$ denotes the log-likelihood contribution of subject i to the full joint mixed model.

3.3.1 Pairwise Modeling Approach

Because of computational complexity of joint modeling, the Pairwise Modeling proposed by Steffen Fieuws and Geert Verbeke is used [12]. This is obtained by fitting first all pairwise bivariate models separately, instead of maximizing the likelihood of the full joint model.

Let m be the number of outcomes that need to be modeled jointly. In the case of PRAQ, $m = 5$, let Y_{ri} denote the $r = 1, \dots, m-1$ to be the indicator of a particular outcome.

Then loglikelihoods of the form $\sum_{i=1}^N l_{rsi}(Y_{ri}, Y_{si} | \Theta_{r,s}) \equiv \sum_{i=1}^N l_{pi}(\Theta_p)$ where $r = 1, \dots, m-1, s = r+1, \dots, m$ and with a N total number of subjects. $\Theta_{r,s}$ stands for the vector of parameters in the bivariate joint mixed model corresponding to these r, s pairs. The second part of the previous equation denotes the pairs “ s, r ” as “ p ”. Finally let Θ represent the stacked vector combining all pair-specific parameter vectors Θ_p . Estimates for the elements of Θ are obtained by maximizing each of the P likelihoods separately. Assuming the full joint model is correct, all possible pairwise models are correct. In the context of the maximum likelihood estimation, each pairwise model has consistent estimates with asymptotic normality.

After fitting all pairwise bivariate models, the obtained estimates are averaged to obtain a single estimate for each parameter in Θ^* . These averages are asymptotically normally distributed with the correct parameter values as mean. In the case of the standard errors further refinement should be done.

Two pair-specific estimates corresponding to two pairwise models with a common outcome are based on overlapping information and hence correlated. In order to account for the sampling variability of the combined estimates in the parameter estimates of Θ^* , consider $\hat{\Theta}^*$. With a pseudolikelihood approach, a covariance matrix for the elements of $\hat{\Theta}$ is constructed [13]. The

idea is to replace the joint likelihood by a suitable product of marginal or conditional densities. Then fitting all pairwise models is equivalent to maximizing a pseudo-likelihood function of the form:

$$pl(\Theta) = l(Y_1, Y_2 | \Theta_{1,2}) + \dots + l(Y_{m-1}, Y_m | \Theta_{m-1,m}) = \sum_{i=1}^p l_p(\Theta_p)$$

since the pairwise approach fulfill the pseudolikelihood framework, and asymptotic multivariate normal distribution for $\hat{\Theta}^*$ is obtained in the form

$\sqrt{N}(\hat{\Theta} - \Theta) \sim MVN(0, J^{-1}KJ^{-1})$ with $J^{-1}KJ^{-1}$ being the variance- covariance matrix. As said before, averaging all pairs of estimates is needed. The estimates for the parameters in Θ^* are calculated using a suitable matrix A of coefficients as follows: $\hat{\Theta}^* = A\hat{\Theta}$. $\hat{\Theta}^*$ follows a MVN distribution with mean Θ^* and covariance matrix $A\Sigma(\hat{\Theta})A'$. $\Sigma(\hat{\Theta})$ is the covariance matrix obtained by $MVN(0, J^{-1}KJ^{-1})$.

3.4 Parameter Estimates

Parameter estimation in GLMMs typically involves maximum likelihood (ML) or variations of ML. Additionally, the solutions are usually iterative ones that can be numerically quite intensive. Here, the solution is merely sketched; further details can be found in [11]. For models presented for ordinal and count data indicate the probability of a level-1 response Y_{ij} for a given subject i at timepoint j , conditional on the random effects b_i . While the form of this probability depends on the form of the response variable, let $P(Y_{ij} | b_i)$ represent the conditional probability for any of the GLMMs for ordinal or Poisson previously defined. For simplicity we omit conditioning on covariates x_{ij} . Let Y_i denote the vector of responses for a particular subject i . The probability of any response pattern Y_i with size n_i conditioned on the random effects, is equal to the probabilities of the level-1 responses:

$$l(Y_i | b_i) = \prod_{i=1}^{n_i} P(Y_{ij} | b_i)$$

The assumption that a subject's responses are independent given the random effects (and therefore can be multiplied to yield the conditional probability of the response vector) is known as the conditional independence assumption. The marginal density of Y_i at population level is expressed as the following integral of the conditional likelihood

$$h(Y_i) = \int_{b_i} l(Y_i | b_i) f(b_i) db_i$$

$f(b_i)$ represents the density function of the random effects, assumed to be a normal density. The marginal log-likelihood from the sample of N subjects is then obtained as $\log L = \sum_{c=1}^N \log h(Y_i)$. Maximizing this log-likelihood the ML estimates are obtained. The integral $h(Y_i)$ has to be integrated using numerical procedures. Adaptive Gauss–Hermite quadrature with 20 quadrature points was used to fit the models.

Direct Likelihood GLMM Poisson model was fitted using R software version 0.5.7 with the package lme4 and Direct likelihood GLMM ordinal models were fitted using SAS proc NLMIXED version 9. The Multiple Imputation GLMM Poisson model was fitted using package MICE and the Multiple Imputation GLMM ordinal analysis was done by considering Multiple Imputation with *Proc mi* in SAS. Direct Likelihood was used under Missing at Random MAR Ignorable assumptions.

4. Results

Direct Likelihood GLMM was used at individual level to determine whether changes in depression or anxiety in participants (measured by EPDS and PRAQ respectively) were affected by changes in the diurnal cortisol pattern while conditioning for variables like age, STATE anxiety, having lost a child in the past, smoke status having children in the past and ever having health problems.

4.1 Poisson GLMM for depression

Hierarchical generalized linear mixed modeling (GLMM) was used to determine whether temporally (by trimesters) changes of Depression (measured by EPDS questionnaire) within participants were accounted by their averaged levels of cortisol at individual patient level. It was also of interest to study the variation of the anxiety and depression in individuals during pregnancy.

GLMM methods were employed to model depression since they allow exploring individual profile differences that come from the variability within individuals (Figure. 1) and also yielding to unbiased estimates while taking the missing nature of the data into account.

Firstly, a quadratic variance structure was tested in model [1] by means of chi-square (p-value 2.2e-16), it was seen that keeping the random time effect increased the likelihood of the parameter estimates from -401 to -357.2 in this model. Then, by means of manual stepwise procedures, the model was reduced testing the deletion of each variable with the t-test. The limit alpha for removing a variable was 0.20 (cortisol and trimester variables are retained a priori). The final model obtained by this procedure (fitted using Direct Likelihood) is shown in Table 6.

$$\log EPDS_{jk} = \beta_0 + u_{01}t_{jk} + \beta_1Cortisol_{jk} + \beta_2Anxiety(STATE)_{jk} \quad (1)$$

β_0 is modeled by:

$$\begin{aligned} &\gamma_{00} + u_{00} + \gamma_{01}Trimester2 / 1 + \gamma_{02}Trimester3 / 1 + \\ &\gamma_{03}Have_Children / not + \gamma_{04}Not_Lost_Child / yes + \gamma_{05}Smoking / not + \\ &\gamma_{06}Age + \gamma_{07}Health / not \end{aligned}$$

$$\text{With } \begin{bmatrix} u_{00} \\ u_{01} \end{bmatrix} \sim N(0, \Sigma) \text{ where } \Sigma = \begin{bmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{12} & \sigma_2^2 \end{bmatrix}$$

Model (1) was used as the saturated model; the random intercept and slope u_{00} and u_{01} , normal distributed with mean zero and unstructured variance covariance matrix Σ , were included in the model to allow the effects of trimester to vary randomly from subject to subject. The reason for

this consideration was because it's being of interest to model intrasubject variability of depression and it's not being constant (the variance of EPDS per trimester was 17.54, 15.64 and 20.94 respectively see Table 3), indicating that a good approach could be to consider a random intercept and a random slope.

Cortisol is the averaged measure of cortisol in saliva for trimester *j* and patient *k*. *Have_Children / not* represents the variable Having children Vs. not having children; *Not_Lost_Child / yes* represents the variable that indicates if the participant have ever had lost a child at any age, *Smoking / not* represents the indicator of smoking Vs. not smoking and *Health / not* represents the variable that indicates if the patient have ever had psychological problems (treated of anxiety or depression) or has ever been diagnosed with a diseased (cardiovascular, lungs or other diseases) Vs. always being healthy. Stepwise procedures were used to identify the most meaningful variable.

Table 6. Parameter estimates EPDS Depression.

Generalized Linear Mixed Model				
	Estimate	Std.Error	z.value	Pr(> z)
Intercept	0.169075	0.179516	0.942	0.190
Trimester2 Vs. Trimester1	-0.185471	0.055637	-3.334	0.000 **
Trimester3 V. Trimester1	-0.066971	0.061071	-1.097	0.273
Cortisol	-0.00138	0.005055	-0.273	0.780
Anxiety(STATE)	0.039217	0.004124	9.511	0.000 ***

Note: Effect sizes were derived using the following formula of (1-exp(Estimate))x100%				
Signif. Codes	Variance and Correlation random effects			
* p<0.05				
** p<0.01				
*** p<0.001				
	Name	Estimate	Std.Dev.	Correlation
	Intercept	0.3088	0.56	
	Time	0.0007	0.03	0.41

The final multilevel model (Table 6.) revealed a significant effect of the trimester in the levels of depression. Pregnant women shown lower scores of depression (16% of reduction of Trimester2 Vs. Trimester1) in second trimester compared with the first one. There was no statistical difference between the first and third trimesters on depression. Anxiety (STATE) had a positive effect in depression (4% increase per unit of increase in STATE anxiety). Cortisol was not statistically significant. These results (not shown) did not change substantially with 10 imputed datasets (imputating by the Chained Equations Method and then pooling. See section 3.1.1)

4.2 Multinomial GLMM for anxiety

4.2.1 Univariate Modeling

The outcome is itself ordinal, the reason why ordinal statistical models are more indicated. A basic assumption of all commonly used ordinal regression models is that the response variable behaves in an ordinal fashion with respect to each predictor (proportional odds assumption). Assuming that a predictor X is linearly related to the log odds of some appropriate event, a simple way to check for ordinality is to plot the mean of X stratified by levels of Y (denote these by $E(X/Y=y)$). These means should be in a consistent order. If for many of the X s, two adjacent categories of Y do not distinguish the means, it becomes evident that those levels of Y should be pooled [28]. Figure 1B in the appendix show these plots; only the anxiety variable measured by the STATE questionnaire shows clearly ordinality, cortisol on the other hand, does not show ordinality with respect to the last levels of the outcome.

Nevertheless Figure 1B should be interpreted with caution, since the last levels of the PRAQ dimensions contain a very small number of observations as shown in the Table 7.

Table 7. Frequencies According with the levels of PRAQ Questionnaire

Response Levels	1	2	3	4	5	6	7
PRAQ1 (Relationship)	61	44	14	23	9	3	1
PRAQ2 (Child's Integrity)	45	43	35	15	11	4	2
PRAQ3 (Fear Delivery)	72	37	24	13	8	1	
PRAQ4 (Moder's Life)	77	38	16	17	6	1	
PRAQ5 (Relationship child)	95	35	19	5	1		

Then for instance in PRAQ5 dimension there was only one participant (patient 73 from 192 participants) that scored 5 in this dimension. PRAQ5 represents the mother's fear about father-child and partner relationship, therefore it could be said that mothers in general do not fear about father-child and partner relationships as they fear about child's integrity (PRAQ2), since scores of anxiety are higher for the last dimension.

In order to make the outcome variable more ordinal with respect to cortisol, age, anxiety and to have more observations in the last levels, a new recategorization of the levels of PRAQ was made. The levels were kept the same as possible, but levels 5,6,7 of each dimension of PRAQ were grouped. The assumption of proportional odds ratios with the new changes held (p-values of 0.2324, 0.1247 and 0.1456 per trimester respectively using the score test, in the case of PRAQ4 for example). It should be taken into account that this change alters de PRAQ scales interpretation since now the cases in the last three levels for all PRAQ dimensions are now grouped.

Following model was then used as the saturated model using the same model reduction already explained in the previous section.

$$\begin{aligned} \text{logit}[P(PRAQ_{ij} \geq c | b_i)] = & b_i + \gamma_c + \beta_1 \text{Cortisol}_{ij} + \beta_2 \text{Anxiety}(\text{STATE})_{ij} + \beta_3 \text{Trimester2} / 1 + \\ & \beta_4 \text{Trimester3} / 1 + \beta_5 \text{Children} / \text{not} + \beta_6 \text{Health} / \text{not} + \beta_7 \text{Smoking} / \text{not} + \\ & \beta_8 \text{Not_Lost_Child} / \text{yes} + \beta_9 \text{Cortisol}_{ij} \text{Vs. Trimester2} / 1 + \beta_{10} \text{Cortisol}_{ij} \text{Vs. Trimester3} / 1 \\ c = & 1, 2, 3, 4, 5 \\ b_i \sim & N(0, \sigma^2) \end{aligned} \quad (2)$$

Only a random normal distributed intercept with mean zero and a variance parameter to be estimated was used. The reason is that the variance is constant overtime for all dimensions (for instance PRAQ1 had a variance equal to 2.11, 2.269 and 2.095 in each trimester respectively see Table 4). $PRAQ_{ij}$ represents each one of the measures of the PRAQ dimensions for a particular time i from a particular subject j. β_1 , β_9 and β_{10} represent the effect of cortisol on the anxiety subscale for a given trimester versus first trimester. The reason for using three parameters instead of one comes from the differences in the means of cortisol per trimester (see Table 5).

The final results obtained from model (2) for a particular PRAQ dimension (fear of integrity of the child) are presented in 9. This is the only dimension of PRAQ were cortisol was statistically significant. The final models of other dimensions are available in Table 9.A in the appendix.

Table 9. Direct Likelihood Ordered Regression estimates for PRAQ2

Direct Likelihood Parameter Estimates PRAQ 2				
Solutions for Fixed Effects				
Effect	Estimate	StdE	t Value	Pr > t
Intercept 5	-3.92	1.00	-3.94	0.0001
Intercept 4	-1.85	0.98	-1.88	0.0604
Intercept 3	0.08	0.98	0.08	0.9367
Intercept 2	1.95	1.00	1.96	0.0509
Trimester1 Vs. Trimester3	-2.84	0.85	-3.33	0.001
Trimester2 V.s Trimester3	-1.68	0.54	-3.12	0.002
cortisol	0.07	0.05	1.34	0.1819
Having Vs Not having children	-1.34	0.38	-3.49	0.0005
Anxiety-STATE	0.07	0.02	3.42	0.0007
Cortisol*Trimester 1 or 2/3	-0.06	0.02	-2.6	0.0098
Variance Random Intercept				
	Estimate	Std E.		
	6.6747	1.4339		

The ordered probability in model (2) is modeled in terms of log odd ratios. This means that the interpretation is given in terms of odds ratios; for instance, Table 9 shows that the ordered log

odds effect of previously having children in comparison to the group of participants that never have never had children on the fear of the baby's integrity scores is -1.34. This is the proportional log odds ratio of women having children (one or more than one) in comparison with women without having children in the past. In other words, the odds of having higher mean levels of anxiety related with the integrity of the baby in a pregnant mother is 0.26 times the odds of women without children. Therefore, a pregnant mother is around four times more relaxed about the integrity of her baby than a pregnant woman without children.

A very interesting feature about the model expressed in Table 9 is the variation of the cortisol effect on this PRAQ dimension according to a particular trimester. This was expected (see how means of cortisol vary across trimesters in Table 5) and therefore the interpretation of the effect of cortisol on anxiety should be given by trimesters. For instance, the log odds ratio effect per unit increase of cortisol in the fear about the baby's integrity in first trimester is 1.01 in the first and second trimester and increases to 1.072 in the last trimester; in other words anxiety increase around 1%(7%) per unit of increase of cortisol in the first and second trimester (third trimester). These results imply a proportionality of cortisol on *fear about the baby's integrity* in a particular pregnant woman, having higher PRAQ2 scores for higher cortisol levels, especially in the last period of pregnancy. Notice also that this effect of cortisol is different for different levels of fear in the participants (see for instance Figure 6 in the next page).

There is a significant log odds effect of Trimester1 Versus Trimester3 (Trimester2 Versus Trimester3) on PRAQ2 equal to -2.28(-1.68). This means that the ordered odds of having higher levels of anxiety ,because *their fear of child's integrity*, in the first trimester (second trimester) are around 6% (19%) times the fear in the last trimester of pregnancy.

There is also a highly significant effect of Anxiety-STATE on PRAQ scores. This means that the current psychological status of a particular pregnant woman at the moment of answering PRAQ questionnaires is relevant. The effect of Anxiety-STATE on fear of child's integrity is about 7% increase per unit of increase in this variable. Finally, Table 9 Also shows that a pregnant mother is more likely to be less concerned about the integrity of her baby (odds ratio=0.262 with a 95% C.I [0.124, 0.551]). Then the ordered odds of being in higher levels of anxiety for a pregnant mother is 0.262 times the odds of the pregnant woman without child or children.

Intercepts do not have interpretation, since their interpretation depends on evaluating the covariates at zero and the range of cortisol and Anxiety-State do not contain zero (see the range of cortisol in Table 5). In any case; intercepts are useful to compute response probabilities.

To give an example of the response probabilities $p(Y_{ij} = c | b_i = 0, x_{ij})$ defined in section 3.2.2, Figure 6 gives us an idea of the behavior of the probabilities per each individual level of PRAQ2

in a particular participant with random intercept equal to zero while controlling for Anxiety-STATE (keeping STATE constant by its mean equal to 34.6).

Figure 6. Plot of the predicted probabilities per levels of PRAQ2 (fear of child's integrity) using the reduced model of Table 9. Each curve represents the probability of having higher levels in contrast with lower levels of anxiety, while keeping anxiety-STATE constant.

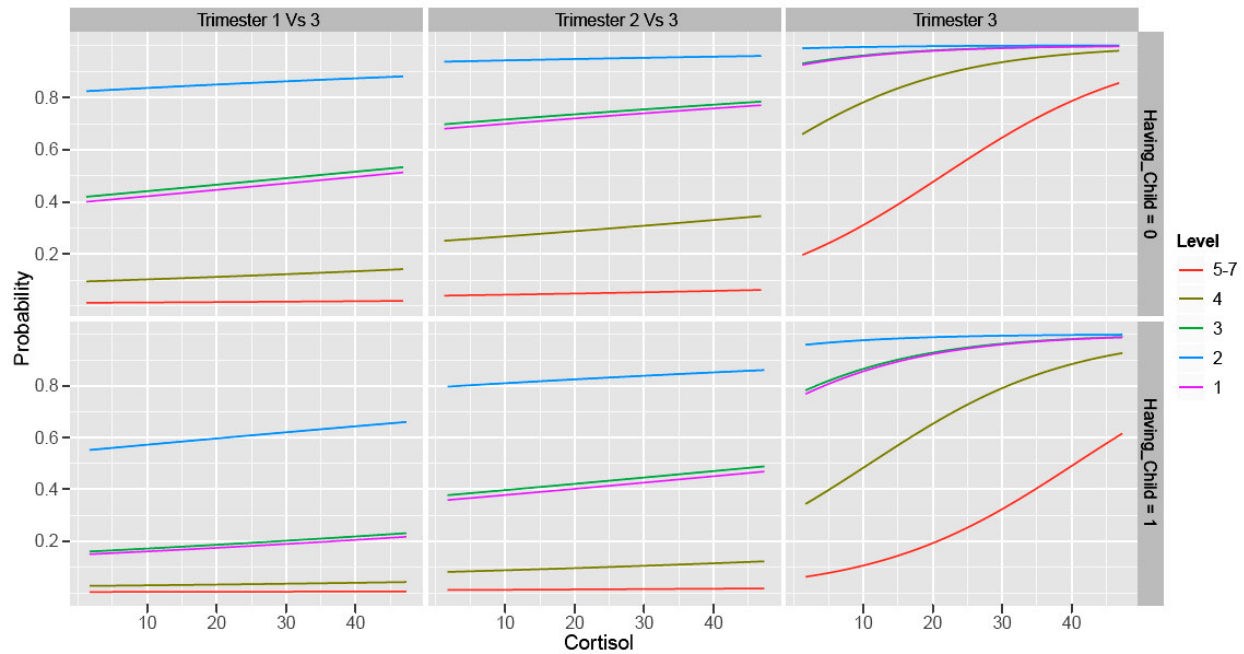


Figure 6 is divided in different panels. In the last two panels labeled with *Trimester 3*, each curve represents the probability of having higher levels in contrast with lower levels of anxiety, while keeping anxiety-STATE constant at its mean. Then, for instance, the red line in the last two panels represent the probability of being high anxious (levels 5-7) versus being middle or not anxious (levels 1-4) about child's integrity in *Trimester 3* of a particular pregnant woman. Notice how having high levels of anxiety becomes more probable as cortisol increase; especially when the pregnant woman is still not jet a mother (last higher panel).

The first four panels represent also the same probabilities, but for *Trimester 1* and *Trimester 2* versus *Trimester 3*. For instance, the red and dark green line indicate the probability of being middle-high anxious (levels 4-7) versus being not anxious (levels 1-3) about child's integrity in *Trimester 1* and *Trimester 2* versus *Trimester 3* of a particular pregnant woman. Because this red line is almost zero, it suggests that probabilities of being middle-high anxious are very high in the last trimester in comparison with the first trimesters. Notice how cortisol has a null effect in these trimesters for these levels of anxiety.

Horizontal and vertical comparisons can be done in these first four panels. Horizontally, comparisons between *Trimester 1* and *Trimester 2* indicate how the probabilities increase, indicating that in the second Trimester anxiety is higher than in the first one; vertically,

comparisons between upper and lower panels indicate differences between parity: having previous children experience (Having_Child=1) reduce the probability of having higher levels of anxiety.

It can be also seen in Figure 6 that for high levels of cortisol the probabilities of having high levels of Anxiety are notoriously increased. It is also worth noticing how cortisol had different effects according to particular levels of anxiety. Based on Figure 5 it can be stated that probabilities of being less anxious groups (levels of anxiety of 1, 2, 3) increase dramatically as cortisol increases in the last trimester. With high levels of cortisol, the probabilities of having higher anxiety levels are higher only in the last trimester (increase in odds ratio of 7% in Trimester 3 versus 1% in Trimester 2 and Trimester 3), when pregnant women are more concern about the integrity of their baby.

4.2.1.1 Sensitivity Analysis MAR mechanism

It was of interest to investigate how variable could be the obtained results under the same assumption of missing mechanism, but imputing 10 datasets and pooling them as explained in section 3.1.1 imputation was made by means of the Markov Chain Monte Carlo (MCMC) method that assumes multivariate normality in of the variables in the model [30]. The same 20 quadrature points were used for all dimensions of PRAQ. Since cortisol was significant only for PRAQ2, Table 10 shows the results obtained using these methods.

Table 10. Shows Parameter Estimates of PRAQ 2 with Pooled StdE Rubin method

Multiple Imputation Parameter Estimates PRAQ 2				
Solutions for Fixed Effects				
Effect	Estimate	StdE	t Value	Pr > t
Intercept 5	-4.25	0.84	-5.05	0.0001
Intercept 4	-2.48	0.85	-2.93	0.0038
Intercept 3	-0.88	0.83	-1.06	0.2905
Intercept 2	0.78	0.85	0.91	0.3653
Trimester1 Vs. Trimester3	-1.66	0.73	-2.28	0.0248
Trimester2 V.s Trimester3	-0.93	0.48	-1.92	0.0602
cortisol	0.03	0.05	0.65	0.5154
Having Vs Not having children	-1.12	0.30	-3.69	0.0002
Anxiety-STATE	0.07	0.02	3.75	0.0006
Cortisol*Trimester 1 or 2/ 3	-0.03	0.02	-1.7	0.0926
Variance Random Intercept	Estimate			
	3.61			

Under the MAR mechanism, using Direct Likelihood and Multiple Imputation yielded different results with respect to the significance of cortisol at 1% (see Table 9).

4.2.1.2 Sensitivity Analysis: Cortisol Effect

Averaging of cortisol can be seen as arbitrary and results in the analysis could be affected, since this average might result in high or low means according to the available values of cortisol per subject. In order to tackle this issue, the same complete analysis was made with the fourth measure of cortisol per trimester instead of the average. The fourth measure of cortisol is the score of the sample collected 12 hours after participants woke up. This measure was preferable in order to repeat the analysis since it had less missing values (see Table 5). In any case this analysis yielded similar results for EPDS. The estimate of cortisol effect in the reduced model (1) section 4.1 was 0.006424(Std.Error=0.009422 and p-value=0.496). Similar analysis were done with the PRAQ dimensions starting with model (2) but averaging cortisol in its first, third and fourth daily scores, and the estimated cortisol effect on fear *about child's integrity* (PRAQ2) was 0.0044(Std.Error= 0.02758 and a p-value of 0.87) for instance.

4.2.2 Bivariate Multinomial Ordinal Response Modeling

In a second stage of the analysis of the effect of cortisol on anxiety, it was of interest taking PRAQ in its multivariate nature. The GLMMs are joined by specifying a common normal distribution for their random effects. The advantage of this common distribution is that relationships between different dimensions of PRAQ can be considered.

To illustrate the joint bivariate modeling using correlated variance parameters, the joint bivariate model (presented in Table 11A see in the appendix) of the first two dimensions of PRAQ was fitted. Setting zero and non-zero covariances between random effects, the corresponding loglikelihood changed from -1108.65 to -1121.1 . A likelihood ratio test ($\chi^2_1 = 5.2$) rejects the use of two independent models (p-value=0.023). Any of the obtained parameter estimates for the variance of the random intercepts in all the pair-wise models can be found in Table 11.

Table 11. Estimations of the correlations of the random intercepts in the bivariate analysis for different dimensions of PRAQ (PRAQ1 Relationship, PRAQ2 child's integrity, PRAQ3 Fear Delivery, PRAQ4 Mother's life, PRAQ5 Relationship Child)

0.44				
0.52	0.37			
0.38	0.41	0.44		
0.55	0.53	0.72	0.61	

Table 11 shows the correlation of the random intercepts in the bivariate approach, for instance $\rho_{PRAQ1/5} = 0.55$ is the correlation between the random intercepts of the first and last dimensions.

Notice how $\rho_{PRAQ1/5}$ correlation is among the highest correlations. From the point of view of the first and last dimensions, “Concern for oneself and the partner relationship during pregnancy” and “Concern about future mother-child, father-child and partner relationship” respectively, this high correlation seems not to be arbitrary since both dimensions are about anxiety in relationships, then we expect to have high correlations in the random intercepts. In any case, interpreting relationships between outcomes through the correlation of their random effects can be misleading, especially when the calculated correlation between random effects can be over-estimated [31].

A two-stage analysis was performed to verify if the association implied by the joint model was reasonable. The main idea was fitting simple linear regression models $PRAQ_{ij} = \beta_{0i} + \varepsilon_{ij}$ per each subject and for the first and second dimensions of PRAQ (first-stage). The ε_{ij} were considered normally distributed error components. Adding an additional subscript, let β_{01i} and β_{02i} represent the subject specific intercepts for the first and second PRAQ dimension respectively. In a second-stage the correlation between intercepts was obtained from the following model

$$\begin{bmatrix} \beta_{01i} \\ \beta_{02i} \end{bmatrix} = \begin{bmatrix} \beta_{01} \\ \beta_{02} \end{bmatrix} + \begin{bmatrix} b_{01i} \\ b_{02i} \end{bmatrix} \quad (3)$$

The last term in the right-hand side of this equation follows a normal distribution with zero-mean and dispersion matrix Σ . The estimators of β_{01i} and β_{02i} were assumed to follow a normal distribution with mean (β_{01}, β_{02}) and a covariance matrix Σ (more details can be found in [32]).

Notice that there were maximum three measures available per subject and there were also missing values (there were for instance 31, 18, 1 participants with 1, 2, 3 missing values respectively in PRAQ2), then the estimation in any case was not precise. The correlation between the estimators of β_{01i} and β_{02i} was 0.21, with a 95% confidence interval of [0.039, 0.369] (Figures 3A, 4A and 5A show that quarterly correlations between first and second PRAQ dimensions were 0.32[0.17, 0.46], 0.27[0.12, 0.40] and 0.27[0.13, 0.369] respectively). Comparing this correlation with the correlation obtained by the joint model in Table 11 ($\rho_{PRAQ1/2}=0.44$) revealed how the random effects approach overestimated the strength of the associations between the first two PRAQ dimensions in comparison with the calculated two-stage correlation.

Finally, despite the fact that correlations of the random intercepts looked big in general, they could say something about the strength of correlation between all pair-wise PRAQ outcomes. For instance, Table 11 shows a correlation $\rho_{PRAQ3/5} = 0.721$ of random intercepts for the third and fifth dimensions of PRAQ as the highest. It was also observed that in the case of PRAQ3 and PRAQ5 outcomes, their quarterly correlation was the highest among others (0.47[0.35, 0.58], 0.55[0.43, 0.65] and 0.55[0.43, 0.65] in each trimester respectively, see Figures 3A, 4A and 5A in the appendix). But again $\rho_{PRAQ3/5}$ was higher than the two-stage calculated correlation of 0.53[0.394, 0.643].

4.2.3 Joint Multinomial Ordinal Response Modeling

In a final stage of the analysis of the effect of cortisol on anxiety, it was of interest taking PRAQ in its multivariate nature, but now taking the five PRAQ outcomes in a model together using the Pairwise Fitting proposed by Fieuws S and Verbeke G. [12] (see section 3.3.1). This is a high-dimensional random-effect models that can be used without any restriction of dimensionality, it uses all pairwise models, then fitting of the full model is replaced by maximum likelihood estimation of each bivariate model separately. Afterwards, all results are appropriately combined as explained in section 3.3.1.

This method was preferred since it can take advantage of the pairwise fitting done in the previous section and it made possible a more integral approach using available software (NLMIXED with SAS version 9). Obtained results of the variances and correlations are shown in Table 12. It can be observed that, in general, the joint model yield to similar results in the correlations of the random intercepts.

Table 12. Estimations of the variance-correlation matrix of the random intercepts joint analysis for different dimensions of PRAQ (PRAQ1 Relationship, PRAQ2 child's integrity, PRAQ3 Fear Delivery, PRAQ4 Mother's life, PRAQ5 Relationship Child)

7.4					
0.46	5.57				
0.57	0.37	7.18			
0.39	0.38	0.47	4.41		
0.53	0.5	0.67	5.43	5.43	

There were not big changes in the results from the pairwise approach from the previous section in relation with the calculated correlations, but there were changes in the standard errors.

Table 13 shows for instance how the interaction of cortisol and time in trimesters is not longer significant, but in any case, the effect of cortisol is still borderline significant significant 0.056(s.e 0.034).

Table 13. Joint Ordered Regression estimates for PRAQ2

Direct Likelihood Parameter Estimates PRAQ 2				
Solutions for Fixed Effects				
Effect	Estimate	StdE	z value	Pr > z
Intercept 5	-4.721	3.660	-1.29	9.01E-01
Intercept 4	-2.645	3.682	-0.72	7.64E-01
Intercept 3	-0.698	3.950	-0.18	5.70E-01
Intercept 2	1.218	4.086	0.30	3.83E-01
Trimester1 Vs. Trimester3	-2.603	1.481	-1.76	9.61E-01
Trimester2 V.s Trimester3	-1.534	0.793	-1.93	9.73E-01
cortisol	0.056	0.034	1.66	4.84E-02
Having Vs Not having children	-1.328	1.795	-0.74	7.70E-01
Not_Lost_Child/yes	0.904	2.049	0.44	3.30E-01
Anxiety-STATE	0.069	0.032	2.13	1.66E-02
Cortisol*Trimester 1 or 2 /3	-0.054	0.023	-2.35	9.91E-01
Variance Random Intercept	Estimate			
	5.57			

Notice that estimations of the standard errors of intercepts are very big; this was caused because the gradient and hessian should be calculated for each patient in order to estimate the pooled variance covariance matrix and it was not possible to calculate these scores for some patients, therefore the standard errors were calculated from a lower group of observations. For this reason the estimation of the standard errors for the intercepts are unreliable, in any case the estimates are still unbiased. A complete table of the estimates is given Table 12A in the appendix.

5. Discussion and Conclusions

5.1 Review about important factors and variables

It was of interest to establish associations between antenatal maternal anxiety measured with The Pregnancy Related Anxiety Questionnaire (PRAQ) and cortisol, as well as antenatal maternal depression measured with the Edinburgh pregnancy depression scale (EPDS). Univariate as well as multivariate analysis were done.

Univariate Direct Likelihood-Generalized Linear Mixed Models (GLMM) for longitudinal correlated and missing data showed that the odds ratio effect per unit increase of cortisol in the *fear about the baby's integrity* (PRAQ2) in first trimesters of pregnancy is 1.01 and rises to 1.072 in the third trimester. Then higher PRAQ2 scores for higher cortisol levels are expected, especially in the last period of pregnancy are expected.

These results were consistent with the bivariate outcome and the multivariate joint outcome analysis done with correlated random intercepts using the average of the cortisol profile (see section 2.4.3.1). Table 13A (in the appendix) shows that the effect of cortisol on PRAQ2 in the joint multivariate analysis had not significant interaction effect with time, but still the cortisol effect was significant, meaning that the effect of cortisol was constant overtime. Univariate Multiple Imputed –GLMM reported a significant cortisol effect at 10% level of significance (see Table 10 section 4.1.1.1). Other analysis with imputed data showed that cortisol was significant (this analysis was made with 5 imputed datasets, results are not shown), then different conclusions can be drawn when using Rubin's methods to account for missingness.

It was also shown in the univariate analysis that the odds of having higher mean levels of anxiety related with the integrity of the baby in a pregnant mother was 0.26 times the odds of women without children, therefore mothers were more relaxed about the integrity of their babies. These results were consistent with the results in the bivariate analysis but not in the multivariate. It was also noticed that being a pregnant mother had an effect on PRAQ dimensions *concern for oneself and the partner relationship during pregnancy* (PRAQ1), *fear for integrity of the baby* (PRAQ2) and *concern about future mother-child, father-child and partner relationship* (PRAQ5) in the bivariate analysis but not in the multivariate (see a summary in the table 13A in the appendix).

It was also shown that a pregnant woman was more stressed in the third trimester because of her fear of child's integrity than in other trimesters. The odds of being anxious were in the first trimester (second trimester) around 6% (19%) times the fear in the last trimester of pregnancy. On the other hand, the odds of having higher mean levels of anxiety related with PRAQ1, PRAQ2 and PRAQ5 (*concern for oneself and the partner relationship during pregnancy; fear of*

integrity of the baby; and about future mother-child, father-child and partner relationship) in a pregnant woman without having lost a child were 0.26 the odds of a woman having lost a child in the past (for more details see Table 13A in the appendix). Thus having lost a child in the past was a factor that increased anxiety (around four times) in relation with mother-father-child relationship.

Previous results were consistent for the univariate and bivariate analysis but in the joint analysis, *having children or having lost a child*, were not significantly related with the anxiety. There is also a highly significant effect of Anxiety-STATE on PRAQ scores in the univariate and multivariate analysis. In general, it was found that the current psychological status of a particular pregnant woman at the moment of answering PRAQ questionnaires was relevant. The effect of Anxiety-STATE on fear of child's integrity is about 7% of increase per unit of increase in this variable in all performed analysis. A complete summary of the significant estimates of the variable effects can be found in the appendix (Table 13A).

The Multivariate Joint Pairwise Fitting (MJPF) was advantageous, since it was a more general approach and similar results were obtained for cortisol, Anxiety-STATE and for the correlation of the random intercepts. In other applications, the MJPF yielded similar results to the univariate and bivariate analysis [12].

5.2 Review about important relationships in the random effects

Variability of the random effects expresses differences between EPDS/PRAQ among participants. And the estimations were in general high. Notice that in Table 4 (section 2.4.2) the calculated variability is rather smaller than the estimated variability of the random intercepts and the standard error (the variability of the random intercepts in the joint multivariate outcome model was 7.40, 5.57, 7.18, 4.41 and 5.43 for each of the PRAQ dimensions for instance). This was due to the estimates that, in Table 4, did not account for missing data (as shown in Table 4, there were big number of missing values) and the longitudinal nature of the data.

Estimated correlations of the random intercepts in the univariate and multivariate analysis were regarded as high; but in any case were very similar in both analyses. It was noticed how *concern about future mother-child, father-child and partner relationship* (PRAQ5) was highly correlated with all other dimensions (see Figure 3A, 4A, 5A in the appendix, Table 11 in section 4.2.2 and Table 12 in section 4.2.3), suggesting that this dimension can be explained by other PRAQ dimensions of PRAQ.

5.3 Final Remarks

The cortisol effect (per unit of increase) in the odds of *fear about integrity of the baby* was 1% in the first and second trimesters (see Table 9) and 7% in the last trimester. This result goes in line with similar findings in literature [32]. On the other hand, it does not mean that with high levels of cortisol the probability of being anxious become higher, since these probabilities are moderated by the levels of anxiety of the pregnant woman and the pregnancy period. This difference can be subtle but it is nicely clarified in Figure 6 (section 4.2.1).

Missing data in EPDS, PRAQ questionnaires and Cortisol measures, plus the variability of cortisol in the study, cortisol averaging and possible not compliance, could be enough reasons of nonsignificance of cortisol in relation with depression and other anxiety dimension. When the data was imputed (see section 4.1.1.1) or when cortisol was averaged using only at most three of the available measurements (see section 4.1.1.2) or only the last measurement, non-significant results were obtained for all anxiety dimensions.

Furthermore, in other studies, association of cortisol and anxiety were not clear and similar analysis yielded to different findings [2], therefore quality in data collection becomes very relevant. Measuring compliance in the participants is of critical importance, since cortisol varies during the day and during pregnancy quarters (this can also be said for anxiety and depression). There are different methods for checking for compliance, by asking directly or by supporting the quality collection by giving electronic assistants to the participants.

Quality in data collection and planning should by a principle. Planning before data collection is important because relevant objectives and markers can be defined from the beginning making the study more accurate and less annoying for the participants; a good study do not imply having a lot of measured variables per participant, because the quality of the data can be corrupted. Variable planning can become critical to understand the nature of the relationship between anxiety, depression and cortisol. Health indicators for example have been shown to moderate the relationship between anxiety and cortisol [31].

6. References

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7. Appendix

Figure 1A. Distribution of EPDS and its Spearman correlations (with confidence intervals) per trimester. The red curve represents the overall mean.

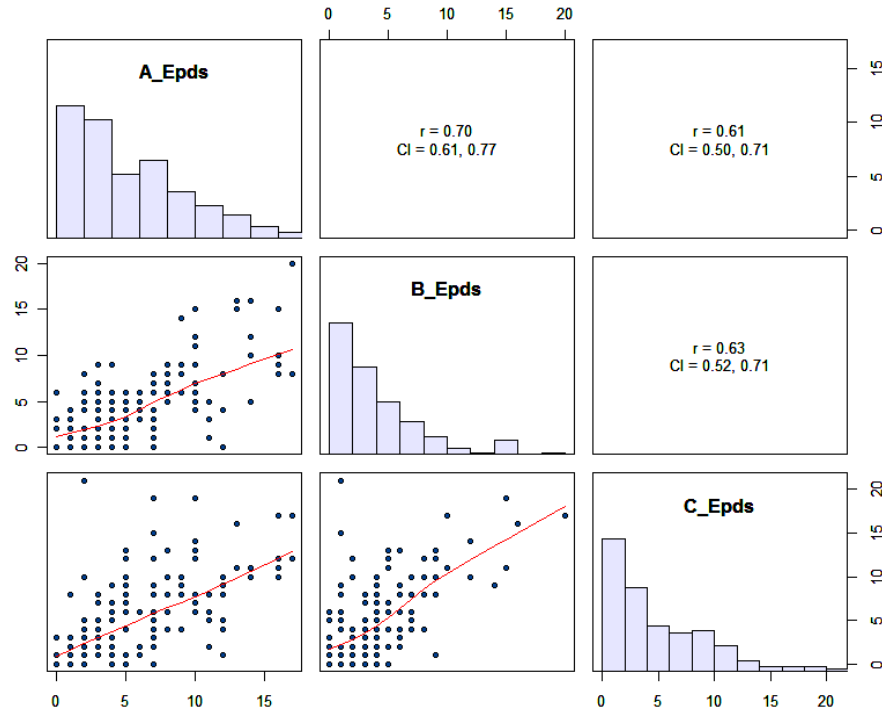


Figure 2A. Distribution of the second PRAQ dimension “fear of the integrity of the baby”, and its Spearman correlations (with confidence intervals) per trimesters.

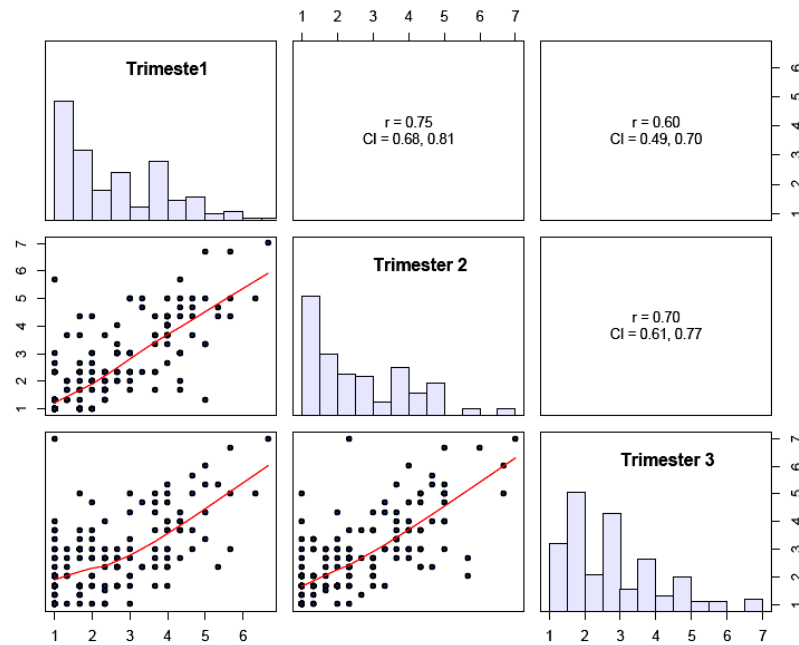


Figure 2.1 A. Distribution of the second PRAQ dimension “fear of delivery”, and its Spearman correlations (with confidence intervals) per trimesters.

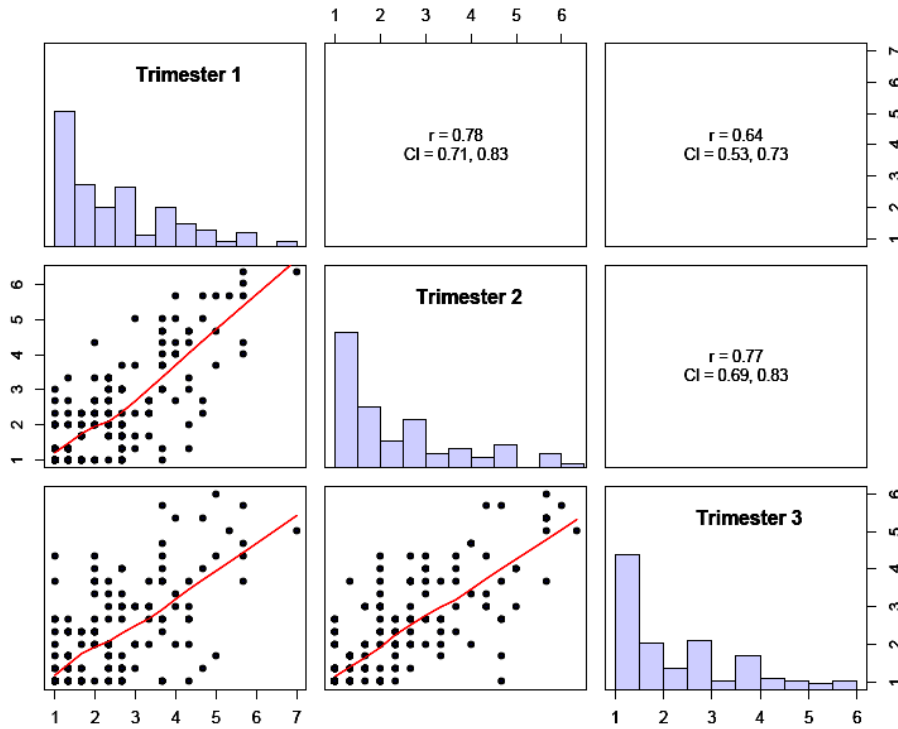


Figure 2.2 A. Distribution of the second PRAQ dimension “fear of changes in the mother's personal life”, and its Spearman correlations (with confidence intervals) per trimesters.

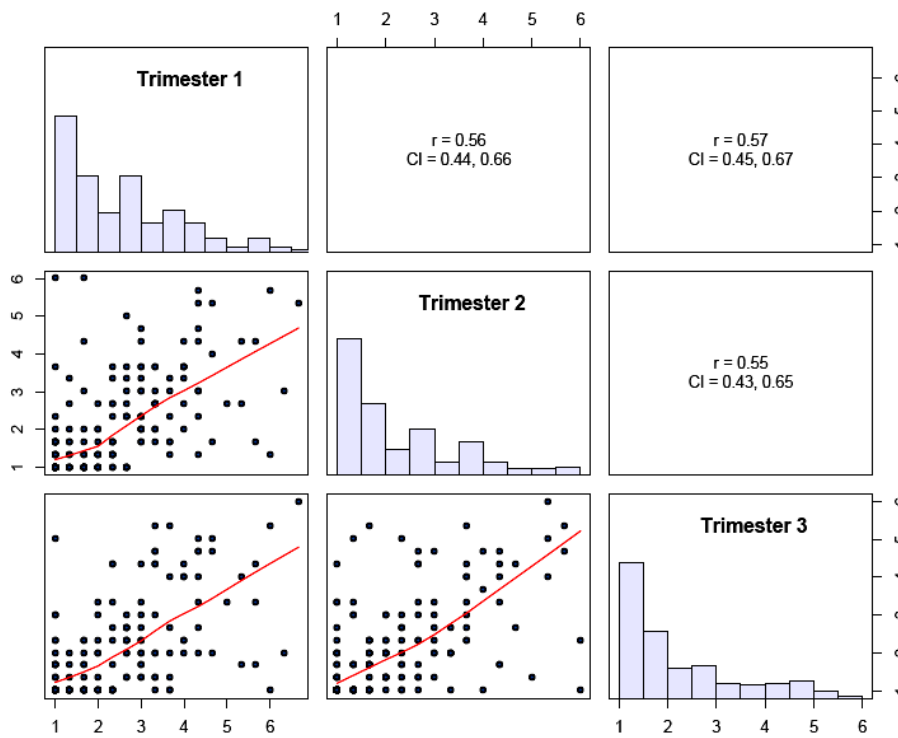


Figure 2.3 A. Distribution of the second PRAQ dimension “Concern about future mother-child, father-child and partner relationship”, and its Spearman correlations (with confidence intervals) per trimesters.

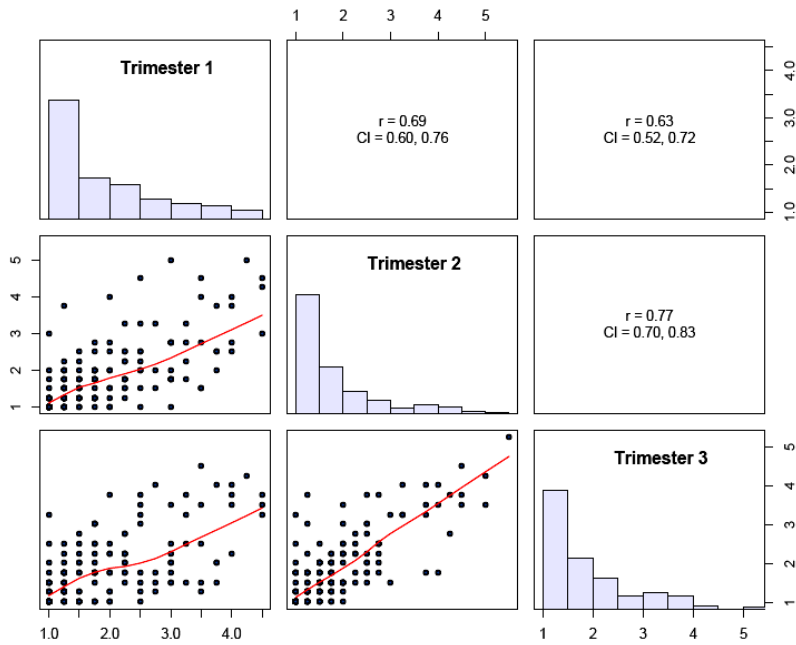


Figure 3A. Distribution of PRAQ dimensions “Concern for oneself and the partner relationship during pregnancy” PRAQ1, “fear of the integrity of the baby” PRAQ2, “fear of delivery” PRAQ3, “fear of changes in the mother's personal life” PRAQ4 and “Concern about future mother-child, father-child and partner relationship” PRAQ5, and its Spearman correlations (with confidence intervals) in trimester 1. The red curve represents the overall mean.

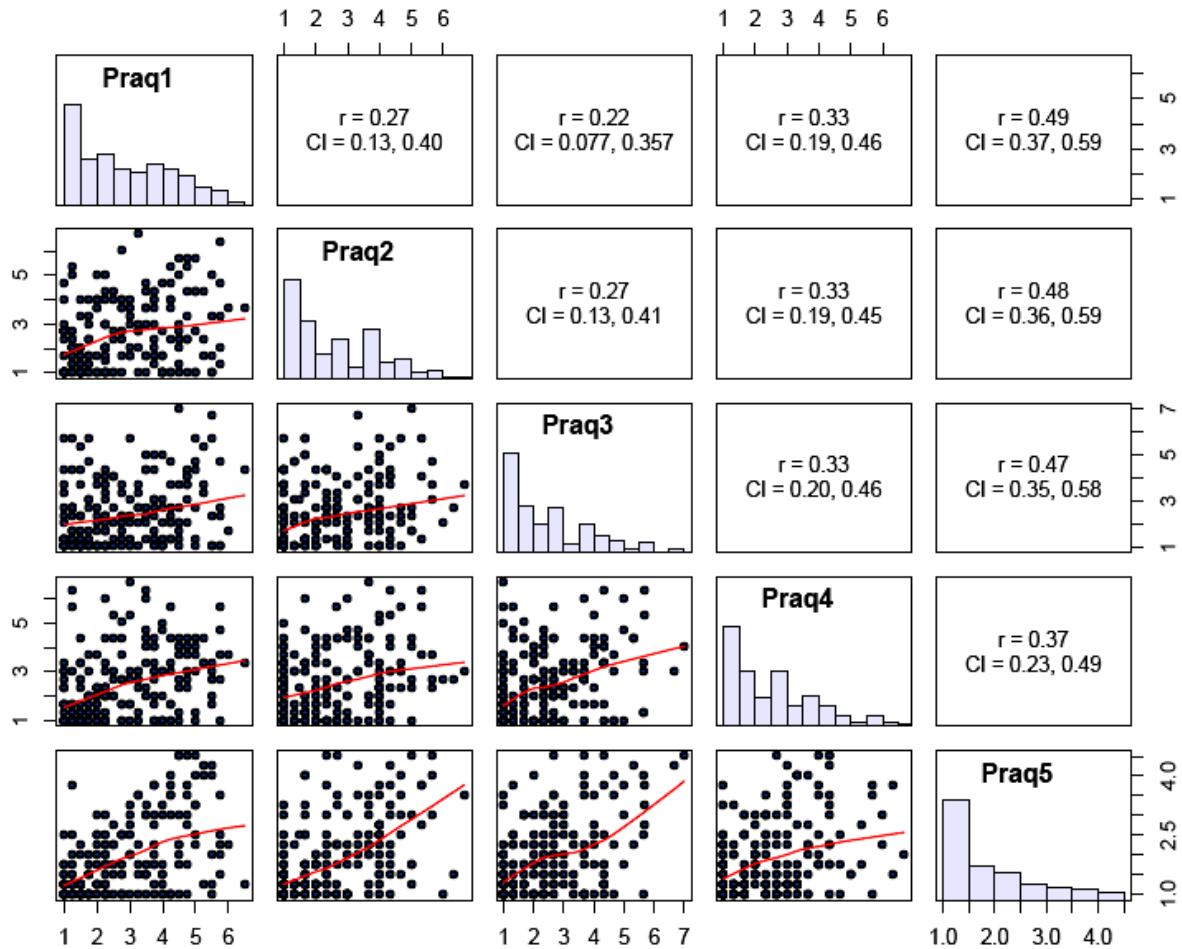


Figure 4A. Distribution of PRAQ dimensions “Concern for oneself and the partner relationship during pregnancy” PRAQ1, “fear of the integrity of the baby” PRAQ2, “fear of delivery” PRAQ3, “fear of changes in the mother's personal life” PRAQ4 and “Concern about future mother-child, father-child and partner relationship” PRAQ5, and its Spearman correlations (with confidence intervals) in trimester 2. The red curve represents the overall mean.

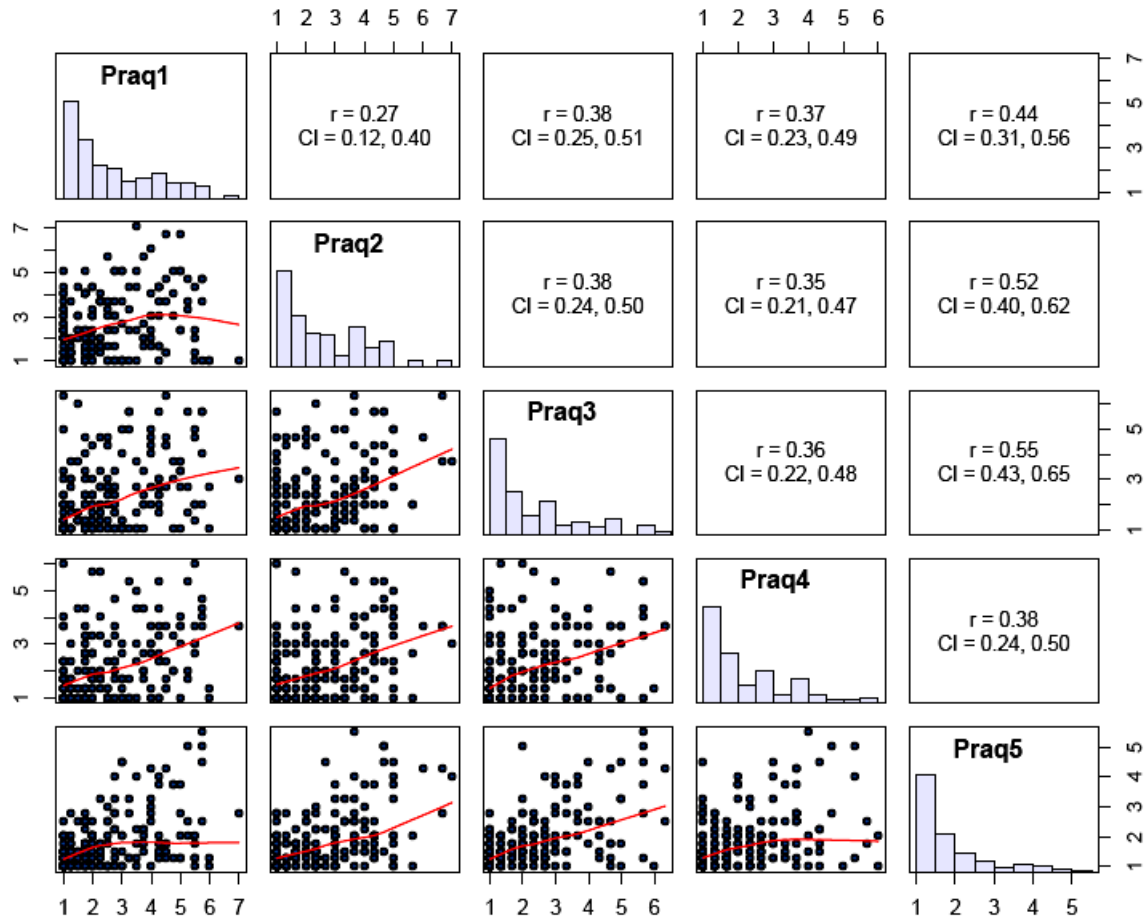


Figure 5A. Distribution of PRAQ dimensions “Concern for oneself and the partner relationship during pregnancy” PRAQ1, “fear of the integrity of the baby” PRAQ2, “fear of delivery” PRAQ3, “fear of changes in the mother's personal life” PRAQ4 and “Concern about future mother-child, father-child and partner relationship” PRAQ5, and its Spearman correlations (with confidence intervals) in trimester 3. The red curve represents the overall mean.

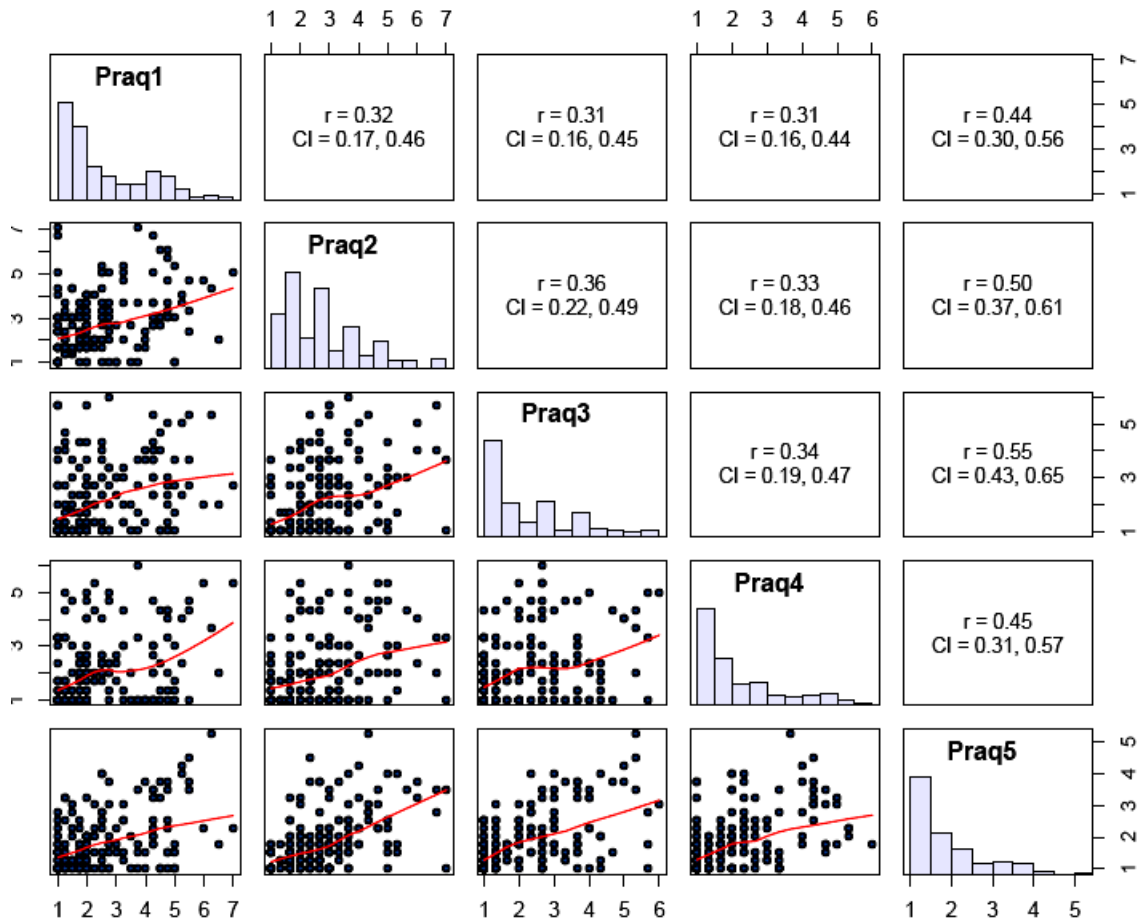


Figure 1B. Plot of the means of selected variables by levels of Y. Stratified means are connected by solid lines and dashed lines connect the estimated expected value of $X/Y=j$ given that PO holds. Estimated expected values from the CR model are marked with c's.

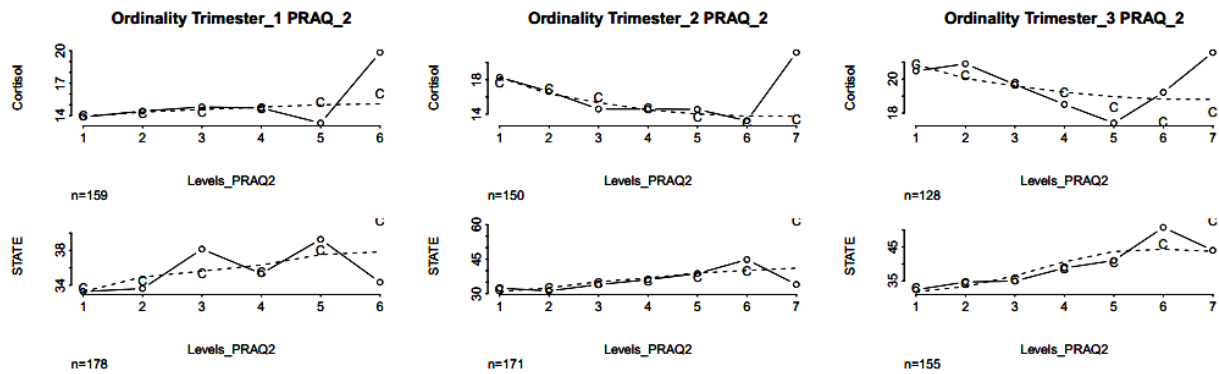


Figure 1C. Cortisol Awakening Response and Hour in which the sample was taken

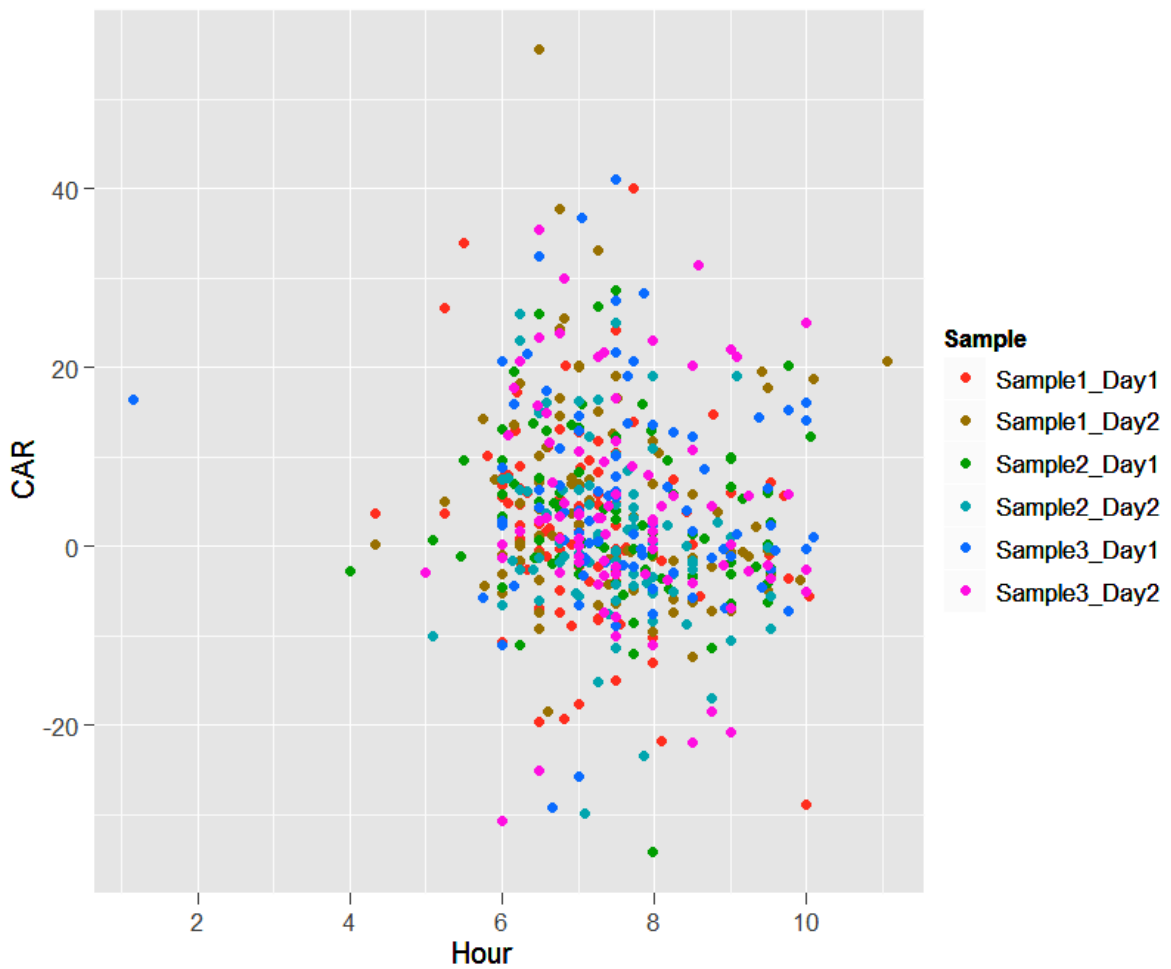


Figure 1C shows the CAR for patients with at least one negative CAR score versus the hour in which the measure was taken. The variable Sample indicates Sample1_Day1 if the measure of CAR took place in the first trimester of pregnancy in the first day; Sample1_Day2 if the measure of CAR took place in the first trimester of pregnancy in the second day. In total there were two measures per trimester and three trimesters. Not important trends are visible and pearsons correlation between the awakening hour and at least one negative CAR scores was of -0.125.

Table 9A. Parameter estimates using Direct Likelihood.

Parameter estimates Univariate Analysis PRAQ1 PRAQ3 PRAQ4 PRAQ4											
	Effect	Int 5	Int 4	Int3	Int2	Trim1Vs.3	Trim2Vs.3	cortisol	Not_CLost	STATE	Var. Comp
PRAQ1	Est	-7.90	-5.75	-4.45	-2.54	1.03	0.42	0.01	-1.33	0.08	8.2
	StdE	0.98	0.94	0.93	0.91	0.29	0.28	0.02	0.54	0.02	1.83
	tVal	-5.45	-3.54	-2.45	-0.81	3.24	1.32	-0.08	-2.46	3.41	
	Pr > t	1E-04	5E-04	0.015	0.419	0.0013	0.187	0.9378	0.0147	7E-04	
PRAQ3	Est	-6.5818	-4.7401	-3.02	-0.87	0.34	0.34	-0.06		0.05	9.15
	StdE	0.98	0.94	0.9	0.88	0.31	0.29	0.025		0.019	1.98
	tVal	-6.7	-5.06	-3.35	-0.98	1.12	1.17	-0.62		2.77	
	Pr > t	1E-04	3E-04	0.009	0.326	0.2615	0.2418	0.533		0.0059	
PRAQ4	Est	-9.82	-8.04	-6.47	-4.75	0.74	0.34	-0.01		0.14	3.34
	StdE	1.01	0.95	0.91	0.88	0.28	0.28	0.02		0.02	0.88
	tVal	-9.76	-8.47	-7.09	-5.39	2.59	1.2	-0.46		7.5	
	Pr > t	1E-04	1E-04	1E-04	1E-04	0.01	0.23	0.6484		1E-04	
PRAQ5	Est	-9.23	-6.22	-3.95	-1.68	0.38	-0.08	-0.03	-2.01	0.07	5.76
	StdE	1.31	1.04	0.96	0.94	0.34	0.32	0.03	0.48	0.02	1.77
	tVal	-7.02	-5.97	-4.09	-1.79	1.12	-0.24	-0.81	-4.23	3.7	
	Pr > t	1E-04	1E-04	1E-04	0.075	0.2654	0.8103	0.4164	0.0001	2E-04	

Table 9A shows the estimates of the effect of Trimester 2 Versus Trimester 3 (Trim2Vs.3), Trimester 1 Versus Trimester 3 (Trim1Vs.3), cortisol, Having never lost a child Vs. ever had lost a child (Not_CLost), Anxiety (STATE) and the estimates of the variance for the random intercepts (first row) and Standard Error (second row). Effect of Cortisol in presented PRAQ dimensions is not significant.

Table 11A. Bivariate estimates allowing for correlation between random intercepts

BIVARIATE ESTIMATES PRAQ 1 AND PRAQ 2								
Parameter	PRAQ 1				PRAQ 2			
	Est.	Std.E.	t	Pr > t	Est.	Std.E.	t	Pr > t
Intercept 5	-6.34	1.30	-4.87	2E-06	-4.99	1.19	-4.188	4E-05
Intercept 4	-4.14	1.33	-3.13	0.0019	-2.85	1.17	-2.443	0.015
Intercept 3	-2.85	1.32	-2.17	0.031	-0.87	1.16	-0.748	0.4552
Intercept 2	-0.92	1.31	-0.70	0.4829	1.14	1.17	0.9712	0.3321
Trimester1 Vs. Trimester3	0.52	0.78	0.66	0.507	-2.68	0.88	-3.042	0.0025
Trimester2 V.s Trimester3	0.14	0.50	0.28	0.7783	-1.56	0.55	-2.809	0.0052
cortisol	0.03	0.05	0.54	0.5883	0.06	0.05	1.1512	0.2504
Having Vs Not having children	-0.04	0.47	-0.09	0.9302	-1.60	0.48	-3.355	0.0009
Not_Lost_Child Vs. Lost_Child	-1.13	0.56	-2.02	0.0439	0.94	0.54	1.7388	0.0829
Anxiety-STATE	0.08	0.03	3.21	0.0014	0.08	0.02	3.7584	0.0002
Cortisol*Trimester	-0.01	0.02	-0.61	0.5416	-0.06	0.02	-2.328	0.0205
Variance Random Intercepts	Var 1	6.51			Var 2	7.02		
	Corr	0.44						

Table 12A. Joint estimates multivariate analysis. Indicator numbers at the end of each variable refer to the PRAQ dimensions to which each estimate belongs to. 1 refers to the concern for oneself and and the partner relationship during pregnancy; 2 refers to the fear of the integrity of the baby; 3 refers to the fear for delivery; 4 refers to the fear for changes; and 5 refers to the concern about future mother-child, father-child and partner relationship.

Parameter	Estimate	StdE	z value	Pr > z
Intercept 2 1	-0.431	5.652	-0.076	5.30E-01
Intercept 2 2	1.218	4.086	0.298	3.83E-01
Intercept 2 3	-0.291	0.715	-0.407	6.58E-01
Intercept 2 4	-3.847	1.660	-2.317	9.90E-01
Intercept 2 5	-1.132	2.360	-0.480	6.84E-01
Intercept 3 1	-2.393	5.633	-0.425	6.64E-01
Intercept 3 2	-0.698	3.950	-0.177	5.70E-01
Intercept 3 3	-2.594	0.756	-3.433	1.00E+00
Intercept 3 4	-5.658	1.630	-3.470	1.00E+00
Intercept 3 5	-3.403	2.466	-1.380	9.16E-01
Intercept 4 1	-3.696	5.936	-0.623	7.33E-01
Intercept 4 2	-2.645	3.682	-0.718	7.64E-01
Intercept 4 3	-4.255	0.732	-5.810	1.00E+00
Intercept 4 4	-7.280	1.644	-4.427	1.00E+00
Intercept 4 5	-5.732	2.864	-2.001	9.77E-01
Intercept 5 1	-5.924	6.405	-0.925	8.23E-01
Intercept 5 2	-4.721	3.660	-1.290	9.01E-01
Intercept 5 3	-13.838	0.771	-17.944	1.00E+00
Intercept 5 4	-18.505	1.627	-11.375	1.00E+00
Intercept 5 5	-14.598	2.752	-5.304	1.00E+00
Not_Lost_Child/yes 1	-1.399	4.492	-0.311	6.22E-01

Not_Lost_Child/yes 2	0.904	2.049	0.441	3.30E-01
Not_Lost_Child/yes 3	-0.458	1.025	-0.446	6.72E-01
Not_Lost_Child/yes 4	-0.220	1.085	-0.202	5.80E-01
Not_Lost_Child/yes 5	0.481	0.746	0.645	2.59E-01
Cortisol 1	0.024	0.352	0.068	4.73E-01
Cortisol 2	0.056	0.034	1.661	4.84E-02
Cortisol 3	-0.093	0.066	-1.412	9.21E-01
Cortisol 4	0.018	0.050	0.366	3.57E-01
Cortisol 5	0.016	0.101	0.160	4.36E-01
Cortisol*Trimester 1	-0.010	0.095	-0.109	5.43E-01
Cortisol*Trimester 2	-0.054	0.023	-2.347	9.91E-01
Cortisol*Trimester 3	0.034	0.029	1.186	1.18E-01
Cortisol*Trimester 4	-0.019	0.017	-1.108	8.66E-01
Cortisol*Trimester 5	-0.016	0.032	-0.508	6.94E-01
Having Vs Not having children 1	0.295	2.868	0.103	4.59E-01
Having Vs Not having children 2	-1.328	1.795	-0.740	7.70E-01
Having Vs Not having children 3	-0.629	0.923	-0.682	7.52E-01
Having Vs Not having children 4	0.226	0.370	0.611	2.71E-01
Having Vs Not having children 5	-1.790	0.379	-4.728	1.00E+00
Anxiety-STATE 1	0.068	0.178	0.381	3.52E-01
Anxiety-STATE 2	0.069	0.032	2.130	1.66E-02
Anxiety-STATE 3	0.038	0.029	1.307	9.56E-02
Anxiety-STATE 4	0.140	0.023	5.967	1.21E-09
Anxiety-STATE 5	0.047	0.034	1.376	8.44E-02
Trimester1 Vs. Trimester3 1	0.607	3.733	0.163	4.35E-01
Trimester1 Vs. Trimester3 2	-2.603	1.481	-1.758	9.61E-01
Trimester1 Vs. Trimester3 3	1.476	0.781	1.889	2.94E-02
Trimester1 Vs. Trimester3 4	0.069	0.584	0.118	4.53E-01
Trimester1 Vs. Trimester3 5	-0.162	0.998	-0.163	5.65E-01
Trimester2 V.s Trimester3 2	0.154	2.421	0.064	4.75E-01
Trimester2 V.s Trimester3 3	-1.534	0.793	-1.934	9.73E-01
Trimester2 V.s Trimester3 4	0.941	0.535	1.759	3.93E-02
Trimester2 V.s Trimester3 5	-0.167	0.373	-0.447	6.73E-01
Trimester2 V.s Trimester3 6	-0.467	0.664	-0.702	7.59E-01

Table 13A. Summary of the performed analysis for PRAQ dimensions. The values that appear are the significant estimates of the GLMM using Direct Likelihood at 5% of significance. P12 stands for the bivariate estimate correlating the random intercepts of the PRAQ dimensions “Concern for oneself and the partner relationship during pregnancy” PRAQ1, “fear of the integrity of the baby” PRAQ2.

Variable		Univariate	Bivariate	Multivariate
Trimester1 Vs. Trimester3	PRAQ			
	1	1.03	P12=-2.68	
	2	-2.84	P23=-2.71, P25=-2.4, P24=-2.5	
	3			
	4	0.74		
Trimester2 V.s Trimester3	5			
	1		P12=-1.55	
	2	-1.68	P23=-1.67, P25=-1.43, P24=-1.5	
	3			
	4			
Cortisol/Cortisol*Trimester	5			
	1			
	2	0.07/-0.06	P12=0.059/-0.05, P23=0.059/-0.06, P25=0.053/-0.05	0.056
	3			
	4			
Having Vs Not having children	5			
	1		P12=-1.6	
	2	-1.34	P23=1.37, P24=-1.06, P25=-1.23	
	3			
	4			
Not_Lost_Child/yes	5		P45=-2.11, P15=-1.46, P25=-1.81, P35=-1.8	
	1	-1.33	P15=-1.32, P13=-1.625, P14=-1.51	
	2			
	3			
	4			
Anxiety-STATE	5	-2.01		
	1	0.08	P14=0.06, P15=0.06, P12=0.08, P13=0.059	
	2	0.07	P23=0.06	0.069
	3	0.05	P34=0.04, P35=0.04	0.038
	4	0.14	P45=0.014, P14=0.13, P24=0.1429	0.14
	5	0.07		0.0437

8. Sample SAS codes

```
PROC IML;

/*create a dataset _TABLEPAIRS with all possible pairs

outcome_nr=J(5,1,0);
N=nrow(outcome_nr);
do i=1 to N;
  outcome_nr[i,]=i;
end;
Npair=N*(N-1)/2;
pairs=J(Npair,2,0);
hulp=1;
do i=1 to (N-1);
  do j=i+1 to N;
    pairs[hulp,1]=outcome_nr[i];
    pairs[hulp,2]=outcome_nr[j];
    hulp=hulp+1;
  end;
end;
c_name = { "n1", "n2" } ;
create _tablepairs from pairs[colname=c_name];
append from pairs;
close _tablepairs;
quit;
data _tablepairs;
format name1 name2 $char14.;
set _tablepairs;pair=_N_;
  if n1=1 then name1="a";if n1=2 then name1="b";
  if n1=3 then name1="c";if n1=4 then name1="d";
  if n1=5 then name1="e";
  if n2=1 then name2="a";if n2=2 then name2="b";
  if n2=3 then name2="c";if n2=4 then name2="d";
  if n2=5 then name2="e";
run;

/*dataset with names of the 5 sets*/
data _sets;format name $char14.;set=_N_;input name;
cards;
a
b
c
d
e
;

/*Importing the dataset*/

DATA rdata ;
INFILE "C:\Users\ELIBIEL\Documents\praq_multi_411.txt"
      DSD
      LRECL= 74 ;
```

```

INPUT
  patid
  resp
  a
  b
  c
  d
  e
trimester
  cortisol
  state
  nchb
  chlb
  age
  ill
  smoke
;
RUN;

*****;
*****STARTING VALUES*****;

%macro pairs;

  /*loop over pairs*/
  %do i=1 %to 10;

    data hulp;set _tablepairs;where pair=&i;
      call symput('n1',n1);call symput('n2',n2);
      call symput('name1',name1);call symput('name2',name2);
    run;
    %let nr1=%trim(&n1);%let nr2=%trim(&n2);

    /*select the data for the i-th pair*/
    data paar;set rdata;where (&name1=1 or &name2=1);run;
    proc sort;by patid ;run;

    /*fit the bivariate generalised linear mixed model*/
    title1 "Pair &i";
    title2 "&name1 and &name2";

    proc nlmixed data=paar qpoints=5 noad maxiter=1000 ;
      parms Int5&nr1 =-2 , Int4&nr1 =-1 , Int3&nr1 =0 , Int2&nr1 =1 , bt1&nr1=0.5
, bt2&nr1=0.5 , bcor&nr1=0.5, bnchb&nr1=0.5,
      bchlb&nr1=0.5, bst&nr1=0.5, bcr&nr1=0.5,
      Int5&nr2 =-2 , Int4&nr2 =-1 , Int3&nr2 =0 , Int2&nr2 =1 ,
bt1&nr2=0.5, bt2&nr2=0.5, bcor&nr2=0.5,
      bnchb&nr2=0.5, bchlb&nr2=0.5, bst&nr2=0.5, bcr&nr2=0.5,
      su&nr1=2.5 su&nr2=2.5 su&nr1&nr2=0.5;

      t1&nr1=(trimester=1); t2&nr1=(trimester=2);
ch&nr1=(chlb=1);nch&nr1=(nchb=1);
      t1&nr2=(trimester=1); t2&nr2=(trimester=2);
ch&nr2=(chlb=1);nch&nr2=(nchb=1);

```

```

        cp5= 1/(1 + exp(-(Int5&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
        Int5&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2)))));
        cp4= 1/(1 + exp(-(Int4&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
        Int4&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2)))));
        cp3= 1/(1 + exp(-(Int3&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
        Int3&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2)))));
        cp2= 1/(1 + exp(-(Int2&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
        Int2&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2)))));

        if      resp=5 then ip = cp5;          /* CP5 is Pr(resp=5) */
        else if resp=4 then ip = cp4-cp5;      /* CP4 is Pr(resp=5 or 4) */
        else if resp=3 then ip = cp3-cp4;      /* CP3 is Pr(resp=4 or 3) */
        else if resp=2 then ip = cp2-cp3;      /* CP2 is Pr(resp=4 or 3 or 2)
*/
        else                                ip = 1-cp2;          /* IP is Pr(resp=observed
level) */

        p = (ip>0 and ip<=1)*ip+ (ip<=0)*1e-8 + (ip>1);
        loglik = log(p);
        model resp ~ general(loglik);
        id cp2-cp5;
        ods output parameterestimates=uit&i;
        run;
data parms&i (keep=parameter estimate);set uit&i;run;

        /*end loop over pairs*/
    %end;

%mend pairs;

%pairs;

```

```

*****;
*****FITTING PAIRWISE*****;

%macro pairs1;

    /*loop over pairs*/
    %do i=1 %to 10;

        data hulp;set _tablepairs;where pair=&i;
        call symput('n1',n1);call symput('n2',n2);
        call symput('name1',name1);call symput('name2',name2);
        run;
        %let nr1=%trim(&n1);%let nr2=%trim(&n2);

        data parmssamen;set parms&i;run;
        /*select the data for the i-th pair*/
        data paar;set rdata;where (&name1=1 or &name2=1);run;
        proc sort;by patid ;run;

        /*fit the bivariate generalised linear mixed model*/
        title1 "Pair &i";
        title2 "&name1 and &name2";

    proc nlmixed data=paar qpoints=5 noad maxiter=1000 ;
        parms / data=parmssamen;

        t1&nr1=(trimester=1); t2&nr1=(trimester=2);
        ch&nr1=(chlb=1);nch&nr1=(nchb=1);
        t1&nr2=(trimester=1); t2&nr2=(trimester=2);
        ch&nr2=(chlb=1);nch&nr2=(nchb=1);

        cp5= 1/(1 + exp(-(Int5&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
        bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
        bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
        bcr&nr1*cortisol*time*&name1+
        Int5&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
        bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
        bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
        bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));
        cp4= 1/(1 + exp(-(Int4&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
        bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
        bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
        bcr&nr1*cortisol*time*&name1+
        Int4&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
        bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
        bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
        bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));
        cp3= 1/(1 + exp(-(Int3&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
        bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
        bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
        bcr&nr1*cortisol*time*&name1+
        Int3&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
        bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
        bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
        bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));

```



```

        cp2= 1/(1 + exp(-(Int2&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
        Int2&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));

        if      resp=5 then ip = cp5;          /* CP5 is Pr(resp=5) */
        else if resp=4 then ip = cp4-cp5;      /* CP4 is Pr(resp=5 or 4) */
        else if resp=3 then ip = cp3-cp4;      /* CP3 is Pr(resp=4 or 3) */
        else if resp=2 then ip = cp2-cp3;      /* CP2 is Pr(resp=4 or 3 or 2)
*/
        else          ip = 1-cp2;          /* IP is Pr(resp=observed
level) */

        p = (ip>0 and ip<=1)*ip + (ip<=0)*1e-8 + (ip>1);
        loglik = log(p);
        model resp ~ general(loglik);
        random theta_&name1
theta_&name2~normal([0,0],[su&nr1*su&nr1,su&nr1&nr2,su&nr2*su&nr2])
subject=patid ;
        id cp2-cp5;
        estimate 'correl' su&nr1&nr2/(su&nr1*su&nr2);
        ods output parameterestimates=uitpair&i;
        run;
data parmspair&i (keep=parameter estimate);set uitpair&i;run;

        /*end loop over pairs*/
%end;

%mend pairs1;

%pairs1;

*****Calculate First and Second Order Derivatives*****;

%macro se;

        /*loop over pairs*/
%do i=1 %to 10;

        data hulp;set _tablepairs;where pair=&i;
        call symput('n1',n1);call symput('n2',n2);
        call symput('name1',name1);call symput('name2',name2);
        run;
%let nr1=%trim(&n1);%let nr2=%trim(&n2);

        data paar;set rdata;where (&name1=1 or &name2=1);run;
        proc sort;by patid ;run;

        /*calculating first and second derivatives*/
proc nlmixed data=paar qpoints=20 noad maxiter=0 start hess ;

```

```

parms / data=parmspair&i;

t1&nr1=(trimester=1); t2&nr1=(trimester=2);
ch&nr1=(chlb=1); nch&nr1=(nchb=1);
t1&nr2=(trimester=1); t2&nr2=(trimester=2);
ch&nr2=(chlb=1); nch&nr2=(nchb=1);

cp5= 1/(1 + exp(-(Int5&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
Int5&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));
cp4= 1/(1 + exp(-(Int4&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
Int4&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));
cp3= 1/(1 + exp(-(Int3&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
Int3&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));
cp2= 1/(1 + exp(-(Int2&nr1*&name1 + bt1&nr1*t1&nr1*&name1 +
bt2&nr1*t2&nr1*&name1 + bcor&nr1*cortisol*&name1 + bnchb&nr1*nch&nr1*&name1+
bchlb&nr1*ch&nr1*&name1+ bst&nr1*state*&name1 +
bcr&nr1*cortisol*time*&name1+
Int2&nr2*&name2 + bt1&nr2*t1&nr2*&name2 +
bt2&nr2*t2&nr2*&name2 + bcor&nr2*cortisol*&name2 + bnchb&nr2*nch&nr2*&name2+
bchlb&nr2*ch&nr2*&name2 +bst&nr2*state*&name2 +
bcr&nr2*cortisol*time*&name2+theta_&name1*&name1+theta_&name2*&name2)))));

if resp=5 then ip = cp5; /* CP5 is Pr(resp=5) */
else if resp=4 then ip = cp4-cp5; /* CP4 is Pr(resp=5 or 4) */
else if resp=3 then ip = cp3-cp4; /* CP3 is Pr(resp=4 or 3) */
else if resp=2 then ip = cp2-cp3; /* CP2 is Pr(resp=4 or 3 or 2) */
*/
else ip = 1-cp2; /* IP is Pr(resp=observed
level) */

p = (ip>0 and ip<=1)*ip + (ip<=0)*1e-8 + (ip>1);
loglik = log(p);
model resp ~ general(loglik);
random theta_&name1
theta_&name2~normal([0,0],[su&nr1*su&nr1,su&nr1&nr2,su&nr2*su&nr2])
subject=patid ;
id cp2-cp5;
by patid ;
ods output hessian=hessian&i parameterestimates=gradient&i;

```

```

        run;
        data hessian&i;set hessian&i;pair=&i;run;
        data gradient&i;set gradient&i;pair=&i;run;
        /*end loop over pairs*/
%end;

%mend se;

%se;

*****;
*****Combine Information from different Pairs*****;

/*3.0. Preliminary Steps

/*stack information estimates, hessian and gradient*/
%macro combine;
    data estimates;set _null_;run;data gradient;set _null_;run;
    %do i=1 %to 10;
        data estimates;set estimates parm&i;run;
        data hessian&i;set hessian&i;run;
        data gradient;set gradient gradient&i;run;
    %end;
%mend combine;

%combine;

/*creation of matrix A used in IML to average over all pairs. A contains
as many
    rows (macro variable NPARAMFULL) as there are unique parameters
*/
%macro createA (data=);
    data A (keep=parameter);set &data;run;
    proc sort data=A;by parameter;run;
    data A;set A;by parameter;
        if first.parameter then output;
        rename parameter=label;
    run;

    %let dsid=%sysfunc(open(A));
    %let nparamfull=%sysfunc(attrn(&dsid,nobs));
    %let rc = %sysfunc(close (&dsid));

    data &data ;set &data;nr=_N_;run;

    %let dsid=%sysfunc(open(&data));
    %let nparampair=%sysfunc(attrn(&dsid,nobs));
    %let rc = %sysfunc(close (&dsid));

    %do j=1 %to &nparampair;
        data hulp;set &data;where nr=&j;call symput('parameter', parameter);run;
        data A;set A;
            if label="&parameter" then par&j=1;else par&j=0;label
par&j="&parameter";

```

```

run;
%end;

data labelA;set A (keep=label);run;
data A;set A (drop=label);run;
data som (keep=som);set A ;som=sum(of _all_);run;

%mend createA;

%createA (data=estimates);

/*3.1. calculate covariance matrix for fixed effects
/* average over all pairs to get the estimates (and SE) of the MMM

%macro average;
proc iml;
  use gradient;
  read all var{gradient} into gradient_i;
  read all var{patid } into id_gradient;
  close gradient;

  %let npair=1;
  %do i=1 %to 24;
    %do j=%sysevalf(&i+1) %to 25;
      use hessian&npair;

      read all var{Int5&i Int4&i Int3&i Int2&i bt1&i bt2&i bcor&i
bnchb&i bchlb&i bst&i bcr&i
Int5&j Int4&j Int3&j Int2&j bt1&j bt2&j bcor&j bnchb&j bchlb&j bst&j
bcr&j su&i su&j su&i&j}
into hessiani&npair;
      read all var{pair} into pair;
      read all var{patid } into id_hessian;
      close hessian&npair;
      %let npair=%sysevalf(&npair+1);
    %end;
  %end;

  nsubjects=192;npair=10;nparameter=25;
  CPgradient=J(npair*nparameter,npair*nparameter,0);
  do i=1 to nsubjects;
    /*subject-specific gradient vector*/
    gradsubject=gradient_i[loc(id_gradient=i),];

    /*cross product of subject-specific gradient vector*/
    crossi=gradsubject*t(gradsubject);
    CPgradient=CPgradient+crossi;
  end;
  Hessian=J(npair*nparameter,npair*nparameter,0);

  %do j=1 %to 10;
    Hessianpair=J(nparameter, nparameter,0);
    do i=1 to nsubjects;
      hulp=hessiani&j[loc(id_hessian=i),];
      Hessianpair=Hessianpair+hulp;
    end;
    %if %sysevalf(&j)=1 %then %do;

```

```

        HESSIAN=hessianpair;
    %end;
    %else %do;
        HESSIAN=block(HESSIAN,hessianpair);
    %end;
%end;

CPgradient=CPgradient#1/nsubjects; /*mean*/
Hessian=Hessian#1/nsubjects; /*mean*/

/*covariance of sqrt(n)*(theta-theta_0)*/
JKJ=ginv(hessian)*CPgradient*ginv(Hessian);

/*covariance of (theta-theta_0)*/
cov=JKJ#1/nsubjects;

use estimates;
read all var{estimate} into estimate;
close estimates;
use A;read all into A;close A;
use som;read all into som;close som;
use labelA;read all into label;close labelA;
A=A#(1/som);
mean=A*estimate; /*mean over effects obtained in different pairs*/
covrobust=A*cov*t(A);
serobust=vecdiag(covrobust)##(1/2);
B=mean||serobust;
parameter=t(label);

/*dataset with final estimates and standard errors*/
create est_pair from B[colname={estimate_pair se_pair}
rowname=parameter];
append from B[rowname=parameter];

/*covariance matrix of all parameters*/
create covariance_robust from covrobust[rowname=parameter];
append from covrobust[rowname=parameter];
quit;

%mend average;

%average;

```


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