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TITLE

Limited association between markers of stress during pregnancy and fetal growth in “Born into Life”, a new prospective birth cohort

RUNNING TITLE

Maternal stress during pregnancy and fetal growth

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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ABSTRACT

Aims: We aimed to investigate the associations between maternal perceived stress or salivary cortisol levels during pregnancy and child birthweight in a new birth cohort.

Methods: Women (n=92) who participated in the LifeGene study and lived in Stockholm were recruited 2010–2012 and followed from before conception, through pregnancy and child birth. The Perceived Stress Scale (PSS) scores and salivary cortisol levels were collected at gestational week 26–28. Birthweight was collected from medical records. Linear regression analyses and Pearson correlations were performed between PSS scores or cortisol levels and birthweight respectively, with adjustment for gestational age.

Results: No significant associations were found between PSS scores or cortisol levels and birthweight. There was a trend towards higher salivary cortisol levels among those with lower birthweight. This effect was attenuated after adjustment for gestational age. Morning cortisol levels ($r=-0.31$, $p=0.01$) and cortisol decline ($r=-0.26$, $p=0.03$) were negatively correlated to PSS scores, and a similar tendency was observed for evening cortisol levels ($r=-0.21$, $p=0.09$).

Conclusion: Maternal stress during pregnancy was not associated to child birthweight. The inverse correlation between PSS scores and cortisol levels may indicate other mechanisms than hypothalamic-pituitary-adrenal axis activity underlying previously described effects of maternal stress on child outcomes.

Keywords: Birthweight, cortisol, fetal development, stress, pregnancy

KEY NOTES

- We studied the relationship between maternal salivary cortisol levels, Perceived Stress Scale scores and child birthweight in a new birth cohort.
- Maternal stress during pregnancy was not associated to birthweight, but the two measurements of stress were negatively inter-correlated.
- These findings indicate that increased hypothalamic-pituitary-adrenal activity may not be the mechanism behind the effects of stress during pregnancy on offspring.

INTRODUCTION

There is growing evidence for the model of fetal programming, indicating that in utero environment influences the development of the fetus, and pathophysiology in the child (1). Research shows that maternal stress during pregnancy predisposes to both premature birth and low birthweight (2–5).

Evidence suggests that the impact of stress on birthweight depends on the type of stressor; perceived stress has less of an impact compared to objective major life events or chronic stress. Also,

pregnancy-specific anxiety in particular has been identified as a risk factor for preterm delivery (5).

In pregnant women, measures of self-reported stress such as the established Perceived Stress Scale (PSS), have not been related to lower child birthweight (6,7).

The major proposed mechanism linking maternal stress during pregnancy to child outcomes is that involving steroid hormones secreted via the hypothalamic-pituitary-adrenal (HPA) axis (8).

During pregnancy, the maternal HPA axis undergoes significant changes with progressive increases in plasma concentrations of corticotropin-releasing hormone, adrenocorticotropic hormone and cortisol (9). Even though the majority of studies suggest that cortisol is a mediator in the relationship between maternal stress during pregnancy and child outcomes (3,8,10,11), the effect of self-reported stress on maternal cortisol levels during pregnancy is still unclear (12). Most studies have found only weak, or non significant associations between self-reported stress during pregnancy, defined using various standardised measurements, and maternal cortisol levels (13). Specifically, studies have also failed to find significant associations between self-reported perceived stress measured using PSS (7,12) or other psychological self-reported measurements of stress (14,15) and maternal salivary cortisol levels during pregnancy.

Despite the lack of evidence for the mediating role of cortisol in the relationship between maternal stress during pregnancy and child outcomes, it is becoming increasingly clear that cortisol plays an important role in fetal development. In a systematic review, Zijlmans *et al* showed that multiple studies have examined the connections between maternal cortisol levels during pregnancy, using various methods of measuring cortisol in plasma, saliva or urine, and physical, cognitive, psychological and cortisol outcomes in the child (16). In respect to birthweight, negative associations have been reported between maternal cortisol levels and child birthweight (17), suggesting that even

though cortisol is important for fetal growth, overly augmented levels during pregnancy may be harmful to fetal development. However, only a few studies have used salivary cortisol as a measurement of cortisol physiology (7,12,18).

In this study we aimed to investigate the associations between maternal stress during pregnancy and fetal growth in the previously undescribed Swedish birth cohort study Born into Life using data collected before, during and after pregnancy. Our three-fold aims were to investigate: the effect of maternal perceived stress on child birthweight, the relationship between maternal perceived stress and salivary cortisol levels, and the link between maternal salivary cortisol levels and birthweight.

METHODS

Study design

This study was part of the prospective longitudinal birth cohort study Born into Life, originally created from the larger LifeGene study (19), and followed a cohort of women before, during and after pregnancy as well as their children in the perinatal and postnatal period (Figure 1). Between the years 2010 and 2012, women who already were participating in LifeGene and living in the Stockholm County Council area were recruited to Born into Life. The goal of combining prenatal, pregnancy and postnatal data was to address questions concerning fetal growth and health outcomes during childhood in relation to early exposure to maternal lifestyle and environment.

In Born into Life, pregnant women answered questionnaires regarding pregnancy, lifestyle and health at gestational weeks 10-14 and 26-28. During pregnancy, they also provided biological material including blood at gestational week 10-14, blood, saliva and faeces at gestational week 26-28 and blood upon admission to antenatal care. Cord blood and placenta samples were obtained from delivery. Blood and faeces were collected in conjunction with the new-born screening test of their child 2-4 days after delivery and faeces samples at six months post-delivery. From delivery, the medical birth records regarding both mother and child were collected from Danderyd Hospital. Children were followed at 6, 12 and 24 months of age with self-reported parental questionnaires and biological material including blood, saliva and faeces from the child.

Inclusion criteria for the mothers in Born into Life were that they had responded to baseline questionnaires from LifeGene, were pregnant, and gave informed consent. They were recruited both before and after gestational week 10-14, but no later than week 26-28. For children, existing inclusion of their mothers in the maternal cohort and informed consent from both parents were the only inclusion criteria.

Ethical approval was granted by the Regional Ethics Review Board in Stockholm, Sweden. Informed consent from both parents was obtained for all study participants.

Study participants

Originally 107 pregnant women were included in Born into Life. For this study, the women were studied during gestational week 26-28 of pregnancy with questionnaires and saliva samples, and their child's birthweight from delivery. Only participants who provided any of the questionnaires or saliva samples from gestational week 26-28 were included (n=92).

Measures

Data regarding smoking during pregnancy were obtained from Born into Life questionnaires administered at gestational week 26-28. Data regarding pre-pregnancy body mass index (BMI), in kg/m², and highest attained educational level, ranging from mandatory secondary school to high school, university or other, were retrieved from LifeGene baseline questionnaires. Maternal age at delivery, in years, was calculated from mothers' date of birth.

In order to measure maternal psychosocial stress, a 10-item version of PSS (PSS-10) was administered in questionnaires to mothers at gestational weeks 26-28. On a five-point Likert scale ranging from never to often, it assesses the frequency of situations being perceived as subjectively uncontrollable or stressful. Total scores were obtained by reversing the scores of the positive questions and summing all ten questions, giving a possible total score ranging from 0 to 40, with higher scores indicating a higher degree of perceived stress (20).

In order to determine cortisol concentrations, as a measurement of maternal stress, saliva samples were collected from pregnant women during gestational week 26-28. Women were instructed to produce one saliva sample the evening before and one sample from the same morning as their visit to the test centre. Participants were asked to chew a swab for one minute, thus saturating it with saliva, and then to store it in a plastic tube, marked with date and time, at standard refrigerator temperature. Upon collection, samples were centrifuged for two minutes, transported at standard refrigerator temperature, and then stored at -20°C . Cortisol levels in saliva samples from both mother and child were analysed at the Centre for Child Research, Stockholm South General Hospital, Stockholm, Sweden, using the standardized radioimmunoassay kit CORT-CT2 (Cisbio Bioassays, Codolet, France) according to the manufacturer's instructions. The detection interval ranged from concentrations of 1 to 100 nmol/L. Inter-assay and intra-assay variations were below 5%. All samples were duplicated and averaged.

Birthweight in grams, head circumference and body length, both in centimetres, were recorded by midwives at delivery and later collected from the mothers' and children's joint medical birth records. From the records, data regarding sex, gestational age in weeks and mode of delivery, defined as vaginal delivery or Caesarean section, were also collected.

Data analysis

The data of all 92 women and children were initially analysed for extreme values and outliers by calculating residuals and Cook's distance. Characteristics of our two study samples were described using frequencies, means, medians and standard deviations for continuous values and number of observations and percentages for categorical values. Both exposure and outcome variables were analysed for normality of distribution. Outliers were excluded including one child with very low birthweight ($<1,500$ g) and the two highest evening cortisol values (117.1 and 117.6 nmol/L). These were not included in any of the descriptive or inferential statistical analyses. Due to skewed distribution, values of morning and evening cortisol levels as well as cortisol decline were logarithmically transformed using natural logarithms with base e. Cortisol decline was calculated by

subtracting evening values from morning values (12). All subsequent analyses using cortisol values were performed with logarithmic values, and, for better interpretation, results were transformed back into the original units by exponentiation. Mean values of morning and evening cortisol levels were compared using a paired t-test.

The associations between maternal stress, measured as both PSS scores and cortisol levels (morning, evening and decline separately), and child birthweight were examined using linear regression analysis, adjusting for gestational age. A Pearson product-moment correlation was applied to determine the associations between measurements of maternal stress. Results with a p value of <0.05 were considered statistically significant. All data analysis was conducted using STATA/IC 14.0 (StataCorp LLC, Texas, USA) for Windows.

RESULTS

Study sample characteristics

The participants' mean age was 32.5 years (± 3.7 SD). The sample represented a highly-educated population with the majority of women (88.5%) having received university-level education. Mean pre-pregnancy BMI was 19.8 (± 2 SD), 28 women classified as underweight and one woman as overweight. During pregnancy, four women reported that they had smoked one or more times. Total PSS scores ranged from 12-38, with higher values indicating more perceived stress. The mean value was 25.4 (± 5.9 SD) and the median value 26 (Table 1). In total, 87 saliva samples from the morning and 85 saliva samples from the evening were analysed for cortisol levels. The two highest evening cortisol values (117.1 and 117.6 nmol/L) were identified as outliers. These were not included in any of the descriptive or inferential statistical analyses. The mean cortisol levels of our sample (Table 1) were 41.3 nmol/L (± 13 SD) in the morning, 10.4 nmol/L (± 3.4 SD) in the evening and 30.2 nmol/L (± 12.2 SD) for the decline from morning to evening. There was a statistically significant mean difference between morning and evening values.

The majority of children (82%) were delivered by normal vaginal delivery and all children were born alive. Mean gestational age (GA) was 40 weeks (± 1.4 SD) with four preterm and three post-term

deliveries. All children were born with a normal birthweight (2,500-4,500 g), apart from one child who had a high birthweight above 4,500 g. Mean birthweight was 3,547 g (± 432.9 SD) (Table 2).

Maternal stress during pregnancy does not predict child birthweight

Results of linear regression analyses (Table 3) showed that neither maternal total PSS scores ($\beta = -5.9$, 95% CI -23.4–11.5) nor salivary cortisol levels (morning $\beta = -22.0$, 95% CI -50.6–6.6; evening $\beta = -24.4$, 95% CI -50.2–1.5; decline $\beta = -15.6$, 95% CI -41.1–9.9) were significantly associated to child birthweight. The direction of the association between cortisol levels and birthweight indicated a trend where higher salivary cortisol levels yielded lower child birthweight. This effect was, however, attenuated after adjustment for gestational age.

Negative relationship between psychosocial stress and cortisol levels

There was a statistically significant negative association between total PSS scores and morning cortisol levels ($r = -0.31$, $p = 0.01$) and cortisol decline ($r = -0.26$, $p = 0.03$), showing that women with higher PSS scores generally exhibited lower morning cortisol levels and a smaller cortisol decline (Figure 2). A similar tendency was observed for evening cortisol levels ($r = -0.21$, $p = 0.09$).

DISCUSSION

In this new birth cohort using data before, during and after pregnancy, we found that total PSS scores, as a measurement of self-reported maternal stress during pregnancy, as well as maternal cortisol levels were unrelated to child birthweight. Morning salivary cortisol levels and cortisol decline, but not evening levels, were significantly negatively correlated to PSS scores.

Our findings of no significant associations between total PSS scores from gestational week 26-28 and child birthweight are in line with the findings of similar studies that have previously examined the specific relationship between PSS as a measurement of self-reported maternal stress and child birthweight (6,7). In contrast to this, a number of reviews have presented evidence for the existence of a link between self-reported maternal stress during pregnancy and child birthweight (2–5). The included studies have used other measurements of maternal stress during pregnancy than PSS,

such as maternal trait anxiety, general health questionnaire, major life events or chronic stress. Given the lack of evidence for a specific association between total PSS scores and child birthweight, compared to significant reports of the effect of other measurements of self-reported stress on fetal growth, it is reasonable to assume that perceived stress is less of a predictor of adverse child outcomes, such as low birthweight, than other markers of distress (5).

The inverse relationship we found between total PSS scores and morning cortisol levels and cortisol decline is similar to the findings of Pluess *et al* who showed that high anxiety levels in early pregnancy (defined as gestational week 10-20) predicted low cortisol levels, measured as a diminished cortisol awakening response (21). In parallel, Obel *et al* showed that women with high levels of pregnancy-specific stress, due to fear of pregnancy complications, had lower morning cortisol levels in early pregnancy (defined as gestational week 14) and higher levels in late pregnancy (defined as gestational week 30), arguing that the response to stress may be dependent on the stage of pregnancy (22). A theory that could explain this described negative relationship between self-reported stress and cortisol levels is the suggested gradual dampening of HPA axis responsiveness throughout pregnancy. Mediated via physiologically high levels of corticotropin-releasing hormone and cortisol in pregnant women (23,24), receptors in the maternal HPA axis system are down-regulated towards later gestation, causing stress to be less effective in triggering an endocrine response (25). However, the current evidence supporting this theory is weak, the timing of the unresponsiveness of the HPA axis during pregnancy has not yet been established, nor has the inter-individual variability of the phenomenon been examined (26). These conflicting results, presented both within our study and within the greater context of previous research, emphasise the importance of not assuming that cortisol is the sole or even main mediator in the relationship between maternal stress during pregnancy and child outcomes, and that there could be multiple alternative mechanisms behind this association (4,14,26,27).

We did not find salivary cortisol levels to be related to child birthweight. This is in contrast to the positive findings of the majority of similar studies who have found associations between cortisol levels and lower birthweight (7,12,16–18). However, the comparison of these studies to our findings is limited due to differences in measurements of salivary cortisol levels, sampling methods, large

inter-individual variability in biological material and the fact that not all studies have reported raw values of cortisol levels. An observation from our results is that the strength of the trend of the negative association between cortisol levels and child birthweight diminished after adjusting for gestational age. Logically, this implies that gestational age is shorter for children exposed to higher cortisol levels, thus leading to lower birthweight. This is in line with studies that have shown an association between maternal stress hormone levels and preterm delivery and shorter gestational periods (28).

Strengths and limitations

A major strength of this new and previously undescribed cohort study was its longitudinal design, allowing maternal data, collected before and during pregnancy, to be linked to data on mother and child from delivery and the perinatal period. Also, measures of exposure used in this study have previously been validated, and used in other studies. For example, salivary cortisol levels reflect the free, unbound and biologically active fraction of cortisol in the blood (29), and the diurnal rhythm of salivary cortisol has been determined throughout pregnancy (30). Other studies have demonstrated the validity of PSS (20). Furthermore, the quality of data was high, originating from reliable sources such as medical birth records. The fact that the study participants had Swedish personal identification numbers facilitated the data collection and linkage of data between sources.

There were also limitations of this study. Firstly, the sample size were small, resulting in limited statistical power. This is highlighted by the wide confidence intervals of the β -coefficients in the regression analyses, covering both positive and negative associations. Furthermore, the sample represented a healthy, homogenous population of highly-educated women, which may have decreased the generalizability of our study. However, despite their overall health, our sample of women exhibited higher levels of both PSS scores and salivary cortisol levels compared to previous studies (20,30), indicating that they were in fact more stressed, both subjectively (PSS) and objectively (cortisol), than a normal population. This increased our incentive to study the presented associations in this sample. Secondly, the collection of saliva samples relied on mothers' self-reporting of adhering to instructions and participants provided only one sample of saliva from morning and evening

respectively. Nevertheless, obtaining two saliva samples during the same 24-hour period from each mother allowed us to compare morning and evening cortisol levels, as well as the decline over the day, and to separately examine their associations with both PSS scores and birthweight respectively. Thirdly, birthweight is an equivocal measurement for fetal growth considering that a child can be born with low birthweight either because it is born too early, or because it is small for its gestational age (SGA). However, in our study, the associations in the results were further weakened after adjusting for gestational age, implicating that children who weighed less at birth were simply born earlier, and not SGA.

Future studies

There are a number of possible recommendations for future studies. Research should focus on ascertaining the role of the HPA axis and gestational timing effects of cortisol levels in the link between maternal stress during pregnancy and fetal development as well as to try to better understand other mechanisms that could explain this link. Concerning the measurement of stress, future studies should aspire to use multiple measurements of circadian rhythm of cortisol such as cortisol awakening response or diurnal decline, measured at several time points throughout the entire pregnancy. Whereas salivary cortisol levels collected during basal conditions are good at measuring disturbances in the circadian pattern of cortisol secretion, measuring cortisol reactivity in response to stressful events would be an appropriate method to add to future studies. Regarding the measurement of fetal growth, future studies should, apart from birthweight, also use other measurements of fetal development, such as intrauterine growth retardation and SGA.

Future studies in Born into Life will have the advantage of a unique study design that enables studying mother and child born into a cohort, with both pre-pregnancy and prenatal maternal data, often supplemented with paternal data, as well as peri- and postnatal data from the mother and child. Our continued work in this cohort will aim to further investigate the associations between maternal risk factors and health determinants, and trajectories of fetal and child growth, as well as maternal immunity and metabolism, prior to conception, throughout pregnancy and in early infancy.

CONCLUSION

In this study, maternal stress during pregnancy was not found to be associated with child birthweight, yet the different measures of stress – maternal perceived stress and cortisol levels – were negatively inter-correlated. Considering this inverse correlation, other mechanisms than HPA axis activity may lie behind the effects of maternal stress on child outcomes.

LIST OF ABBREVIATIONS

BMI body mass index

HPA hypothalamic-pituitary-adrenal

PSS Perceived Stress Scale

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Table 1. Descriptive statistics of the study sample of mothers.

Characteristic (n=92)	n (%)	Mean (\pmSD), Median, (Min–Max)
<i>Demographics</i>		
Age (years)	92	32.4 (\pm 3.5), (23–42)
Highest attained level of education	86	
Mandatory secondary school	0	
High-school	7 (8%)	
University	76 (88.5%)	
Other	3 (3.5%)	
Pre-pregnancy weight (kg)	86	65.2 (\pm 10.2), (48–100)
Pre-pregnancy height (m)	87	168.8 (\pm 5.8), (153.5–184)
Pre-pregnancy BMI (kg/m ²)	86	19.7 (\pm 2), (16.4–27.9)
Underweight (<18.5)	28 (29%)	
Normal	60 (70%)	
Overweight (>25)	1 (1%)	
Smoking during pregnancy	76	
Yes	4 (5%)	
No	72 (95%)	
Alcohol during pregnancy	76	
Yes	6 (8%)	
Only before awareness of being pregnant	36 (47%)	
No	34 (45%)	
Illicit drugs during pregnancy	76	
Yes	0	
No	76 (100%)	
<i>Maternal stress</i>		
Morning cortisol levels	87	41.3 (\pm 13), (16.8–86.7)
Evening cortisol levels	83	10.4 (\pm 3.4), (4–23.4)
Cortisol decline	83	30.2 (\pm 12.2), (-3.2–72)
Total PSS scores	74	25.4 (\pm 5.9), 26, (12–38)
<i>Abbreviations:</i> BMI=Body Mass Index. n=Total sample size. n=Number of observations. SD=Standard Deviation.		

Table 2. Descriptive statistics of the study sample of children.

Characteristic (n=91)	n (%)	Mean (\pmSD), (Min–Max)
Mode of delivery		
Vaginal delivery	75 (82%)	
Caesarean section	16 (18%)	
Gestational age (weeks)		39.8 (\pm 1.4), (35.6–42.4)
Preterm (<37)	4 (4.5%)	
Term	84 (92.5%)	
Post-term (>42)	3 (3%)	
Sex		
Male	55 (60%)	
Female	36 (40%)	
Birthweight (g)		3,547.1 (\pm 419.6), (2,720–4,625)
Body length (cm)		50.6 (\pm 1.9), (45–55)
Head circumference (cm)		34.9 (\pm 1.4), (32–38.5)
<i>Abbreviations:</i> n=Total sample size. n=Number of observations. SD=Standard Deviation.		

Table 3. Maternal stress and child birthweight. Regression analyses between maternal salivary cortisol levels and PSS scores during gestational week 26–28 and child birthweight. The regression analyses were performed with and without adjustment for gestational age.

	Birthweight			
	<i>Univariate</i>		<i>Adjusted for gestational age</i>	
	β	95% CI	β	95% CI
Total PSS score	-5.9	-23.4–11.5	-10.8	-25.7–4.1
Morning cortisol*	-22.0	-50.6–6.6	-15.3	-40.0–9.5
Evening cortisol*	-24.4	-50.2–1.5	-17.5	-40.0–5.0
Cortisol decline*	-15.6	-41.1–9.9	-8.8	-31.4–13.8

* In order to interpret the β -coefficient using log-transformed independent variables, the coefficient is displayed as $\beta \times 0.1 (= \beta \times \log(1.10))$, i.e. the mean difference in expected birthweight associated with 10% increase in (non log-transformed) cortisol levels. The confidence intervals correspond to this β .

Abbreviations: CI=Confidence interval. PSS=Perceived stress scale

FIGURE LEGENDS

Figure 1. Flowchart of the Born into Life study.

Figure 2. Self-reported maternal stress and salivary cortisol levels during pregnancy. Scatterplots with regression lines showing the relationships between total PSS scores and morning (a) ($p=0.01$) and evening (b) salivary cortisol levels ($p=0.09$), and cortisol decline (c) ($p=0.03$) respectively. PSS scores, as a measurement of self-reported stress, and salivary cortisol levels were measured in pregnant women during gestational week 26–28. The logarithmic scale of cortisol levels on the y-axis has been labelled in original units.

Abbreviations: PSS=Perceived Stress Scale.

FIGURES

Figure 1.

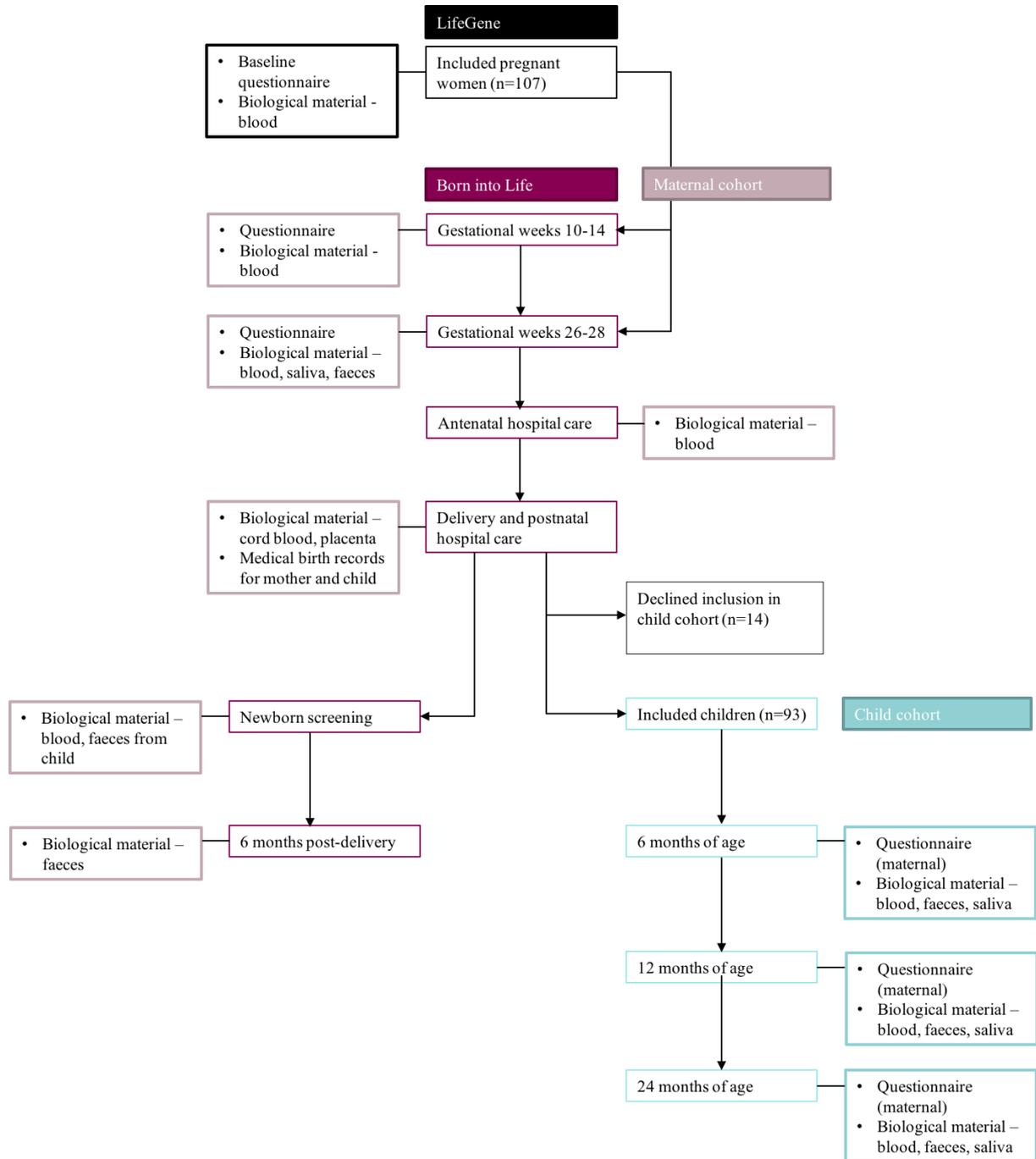


Figure 2.

