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Transcriptomic Signatures of Transition from Late Pregnancy to Postpartum

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Abstract

Background: The peripartum period encompasses physiological, endocrine, and immunological adaptations that ensure maternal health and fetal development. Notably, the molecular correlates of these adaptations in healthy women remain limited, particularly at the transcriptomic level. This study aimed to identify systemic transcriptomic differences between late pregnancy and the early postpartum period in a large cohort of healthy women.

Methods: RNA sequencing was performed on 112 samples from peripheral blood collected at gestational week 38 (gw38; n = 40) and postpartum week 8 (ppw8; n = 72). The transcriptome was compared while adjusting for age and BMI. Additional covariate-adjusted analyses were performed considering cell-type composition, sex of the fetus, and gestational length.

Results: A total of 293 differentially expressed genes (DEGs) were identified when comparing ppw8 vs. gw38, with the majority being downregulated in the postpartum period. The postpartum phase was marked by the lower expression of genes involved in erythropoiesis and innate, granulocyte-driven immunity, as well as higher levels of adaptive, antibody-mediated defense transcripts. The directionality and functional patterns of DEGs were largely preserved, while accounting for shifts in cellular heterogeneity from pregnancy to postpartum, alongside the emergence of DEGs in cell-intrinsic regulatory pathways.

Conclusions: Our results reveal systemic transcriptomic differences from late pregnancy to postpartum, particularly a shift from innate to adaptive immune signatures as well as hematopoietic processes, cellular and metabolic pathways. These findings provide reference data for further understanding of peripartum adaptation and establish a molecular baseline for future investigations into perinatal biology and related disorders.

Keywords: Gene expression; Immune regulation; Postpartum; Pregnancy; Transcriptomics; Peripartum transition; mRNA; hematopoiesis

1. Introduction

The period spanning late pregnancy and the early postpartum phase is characterized by significant physiological and hormonal changes (1, 2). These adaptations include marked dynamics in hematological, immunological, and endocrine systems, which are essential for supporting fetal development, preparing for parturition, enabling successful lactation, and restoring maternal homeostasis following delivery (3, 4). While these adaptations across late pregnancy and postpartum are vital for a healthy delivery and the physiological adjustment of the mother from pregnancy, altered regulation of these processes has been implicated in postpartum complications, including infections, autoimmune disease flares, and mood disorders (5, 6). Therefore, investigating the molecular aspects underlying the adaptive peripartum transition could advance understanding of perinatal physiology, as well as related pathological processes, and assist in identifying diagnostic biomarkers of postpartum health adversities.

To date, research on molecular correlates of pregnancy dynamics has provided insights into physiological immune changes during gestation, such as the increased immune tolerance for the growing fetus compared to a non-pregnant state after delivery (7), and the processes involved in immune dysregulation leading to preterm birth (8, 9). Very few of these studies and with relatively small sample sizes, focused on the transcriptomic level (10-12), reporting upregulation of genes involved in antimicrobial response, immune tolerance, and placental hormone production throughout pregnancy (10, 11). Following delivery, these gestational transcriptomic adaptations appear to revert at different rates across genes and pathways, including a rapid withdrawal of placental hormones and a concomitant shift toward a pro-inflammatory state on the gene expression level (10, 12).

Despite the abundance of transcriptomic studies investigating pathological states and adaptations during the gestational period (11, 13-17), relatively few have focused on the postpartum period in healthy controls (10, 12). These studies particularly lacked the fine resolution across late pregnancy and the postpartum period by focusing on the immediate

postpartum or months after delivery. As a result, the sensitive transition period from pregnancy to postpartum, when adverse health outcomes such as postpartum depression, psychosis or autoimmune flares typically emerge (15, 18), remains poorly characterized. The psychiatric and autoimmune conditions during the postpartum period indeed arise in the early weeks and months after childbirth, with the first 6-8 weeks being more crucial for physiological and psychological adaptation (19-21). Such data, capturing this sensitive postpartum phase, is therefore critical for better understanding perinatal physiology, which will enable delineating the pathophysiology of the perinatal period.

In light of these gaps in the literature, we investigated transcriptomic profiles of healthy pregnant and postpartum women using peripheral blood samples collected at gestational week 38 (gw38) and/or at postpartum week 8 (ppw8) from a perinatal cohort of women in Sweden. Our primary aim was to cross-sectionally identify differentially expressed genes (DEGs) and enriched biological pathways between late pregnancy and postpartum, capturing both system-wide changes, including shifts in blood cell composition, and cell-intrinsic regulatory mechanisms (Figure 1).

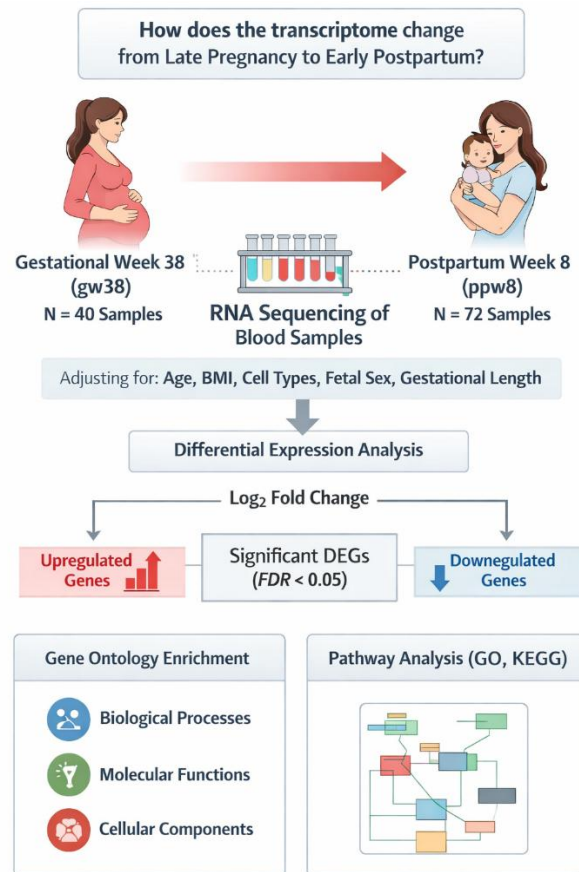


Figure 1. Overview of the study design.

This study presents a transcriptomic analysis of the peripartum transition, to advance understanding of molecular adaptations between late pregnancy and early postpartum in healthy women. Whole blood RNA sequencing was performed in samples collected at late pregnancy (gw38, n=40) and during early postpartum (ppw8, n=72), combining DEGs modeling, multiple testing correction, and gene ontology and pathway enrichment analyses. The inclusion of covariate-adjusted models, particularly accounting for cell-type composition, was implemented to enhance the robustness of the findings. The findings will contribute baseline data for future research in perinatal biology and related disorders.

As a secondary aim, we sought to replicate these findings longitudinally in a subgroup of women providing samples both in late pregnancy and postpartum, to examine within-subject transcriptomic changes.

2. Methods

2.1 Study Population and Biological Sampling

This study is a part of the Biology, Affect, Stress, Imaging and Cognition (BASIC) study, a large, longitudinal, population-based prospective cohort conducted between 2009 and 2019 in Uppsala, Sweden (22). Women undergoing routine ultrasound examination between gestational weeks 16-18 at Uppsala University Hospital were invited to participate in the study. The exclusion criteria for the BASIC cohort were as follows: 1) age under 18 years, 2) non-viable pregnancy at ultrasound, 3) bloodborne infections, 4) difficulty reading or understanding Swedish, and 5) protected identity (i.e., women whose personal data were withheld from public registers and restricted access). The cohort included 6,541 pregnancies in 5,480 women who were followed at gestational week 32, and at 6 weeks, 6 months, and 12 months postpartum. At each of these timepoints, the women completed web-based questionnaires covering sociodemographic details, gynecologic and obstetric information, lifestyle factors, sleep habits, psychiatric history, trauma, stressful events, and other relevant factors. Subgroups of participants were invited for additional visits during gw38 and/or ppw8, at which blood samples were collected. Of the 715 and 713 invitations sent for pregnancy and postpartum visits, respectively, 349 and 413 women consented to participate and provided blood samples.

For the current study, the sample was restricted to participants without severe somatic conditions or clinically significant depression, defined as self-reported depressive symptoms (score of ≥ 13 during pregnancy and ≥ 12 during the postpartum period) at any points assessed by the Edinburgh Postnatal Depression Scale (EPDS) (23), or presence of clinical depression diagnoses or antidepressant use as retrieved from Swedish national health registers. Additional exclusion criteria were: a) failure to provide blood samples, b) twin pregnancy, and c) smoking during the sample collection. After this exclusion, the final cohort included 40 women with RNA samples from gw38 and 72 women with RNA samples from ppw8 (Figure 1). Among the 112 samples collected across the two time points, 12 were contributed by six participants who donated samples at both gw38 and ppw8.

Informed consent was obtained from all participants. This study was approved by the Swedish Ethical Review Authority (Dnr 2009/171) and was conducted in accordance with the Principles of the Declaration of Helsinki (24).

2.2 RNA Sequencing

Peripheral blood samples were collected in PAXgene tubes and stored at -80°C until RNA extraction. RNA was extracted using the PAXgene 96 Blood RNA Kit (Qiagen GmbH) and subsequently prepared for sequencing with the QuantSeq 3' mRNA-Seq Library Prep Kit FWD (Lexogen). The RNA libraries were sequenced using 1 x 100 bp reads on the Illumina NovaSeq 6000 S2 Flow Cell System. Preprocessing of the raw RNA-Seq reads included removing adapters and low-quality sequences using *BBDuk* function from *BBTools* version 39.01 (25) with the following parameters: *k=13*, *ktrim=r*, *usesortkmers=t*, *mink=5*, *qtrim=r*, *trimq=10*, *minlength=20*. Alignment to the UCSC Genome Browser hg38 was performed using Spliced Transcripts Alignment to a Reference (*STAR*) with standard parameters (26). Finally, gene-level counts for each sample were generated using the *htseq-count* function from *HTSeq* library (27). Data have been deposited at the Gene Expression Omnibus (GEO) database repository with the GEO Accession code GSE290313 (15).

2.3 Statistical Analyses

Data quality was assessed by analyzing the distribution of raw counts per sample, requiring a minimum of 25 raw counts across at least 10 samples for between-subject analyses and 25 across 4 samples for within-subject analyses, and by identifying potential outlier genes using Cook's distance. Similar to our previous work (15), differential expression analysis was performed on normalized counts, yielding lists of DEGs with log₂-fold changes (log₂FC) between the compared groups. Negative binomial generalized linear models were used to ensure an unbiased estimation of the net effects of biological factors. Statistical significance was assessed using p-values derived from Wald's test, with corrections for multiple comparisons applied via the Benjamini-Hochberg (BH) method (False Discovery Rate,

FDR). Significant DEGs were determined by applying a fold-change cut-off and significance threshold after BH correction (adjusted p-value (FDR) < 0.05 and $|\log_2FC| > 1$).

After finding significant genes, Gene Ontology Enrichment Analysis (GOEA) was performed only on protein-coding genes to identify enriched biological processes (BP), molecular functions (MF), and cellular components (CC). GOEA was applied to BP, MF and CC ontologies in order to classify and annotate the genes with the *enrichGO* function in the R package *clusterProfiler* (28). Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway overrepresentation analyses were carried out using *enrichKEGG* from the previous R package. Gene ratio was calculated by dividing the number of genes from the dataset in a GO term by the total number of genes with a GO annotation.

Transcriptomic data analysis was conducted in the R environment (ver. 4.3.1)(29) using RStudio IDE (ver. 2023.12.1 Build 402)(30). Tools employed included *DESeq2* (ver. 1.40.2)(31) for variance stabilizing transformation (VST) data normalization and differential expression analysis, *clusterProfiler* (ver. 4.8.2) for GOEA, *biomaRt* (ver. 2.66.2) (32) for accessing ENSEMBL BioMart for functional annotation, and *rrvgo* (ver. 1.24.0) (33) for semantic reduction of GO based on their pairwise similarity, with results visualized as treemap.

Age and pre-pregnancy BMI were included as covariates in all analyses, as these factors could influence both the transcriptome and the study outcomes (34-37). *CIBERSORTx* and LM22 signature matrix were used to compute cell compositions (38). Principal component analysis (PCA) was performed to condense all the estimated leukocyte proportions into principal components (39), thus the first two principal components were added as covariates, including sex of the fetus and gestational length. Gestational length was included as informative for preterm birth due to its known association with transcriptomic variation (8, 10), while cell-type PCs accounted for substantial peripartum changes in blood cell composition. Sex of the fetus was included to account for hormone-related changes across the peripartum period. To test within-subject transcriptomic changes, an additional paired differential expression analysis was performed in six women who contributed samples at both late

pregnancy and postpartum, hereafter called the paired or within-subject analysis. DEGs passing significance criteria of $FDR < 0.05$ and $|\log_2FC| > 1$ in any of three models (age and BMI-corrected, further corrected and paired models) were represented in Supplementary Table 1.

To evaluate the impact of further adjustment on differential expression estimates, we compared the DEG analyses between the two cross-sectional models, that is the age and BMI-corrected model (main model) and the model including cell-type principal components, fetal sex and gestational length (fully adjusted model). For each gene passing the significance criteria mentioned above, $\log_2$ fold-change ($\log_2FC$) estimates were extracted and the effect sizes were assessed using three complementary metrics: the proportion of genes with preserved the $\log_2FC$ direction between models, Spearman's rank correlation coefficient between the two $\log_2FC$ vectors, and a linear regression of fully adjusted the $\log_2FC$ on the main $\log_2FC$, with the slope interpreted as the average shrinkage factor.

3. Results

Individuals were healthy women between the ages of 22 and 42 years, with a group at gw38 being slightly older (mean age 33 years) than at ppw8 (mean age 31 years), but no significant differences in BMI across the two groups (Table 1, $p = 0.04$ for age).

Table 1: Sample Characteristics

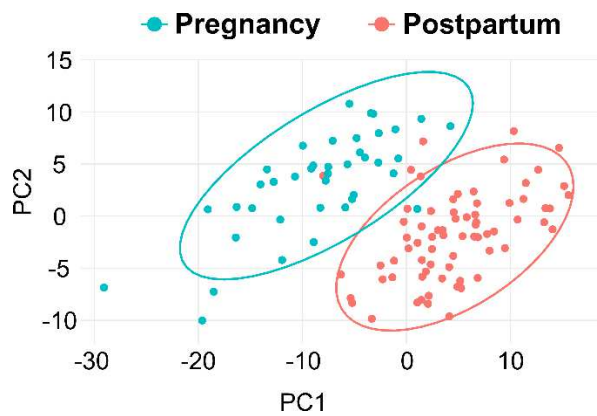
Characteristic	GW38 (N = 40)	PP (N = 72)	p-value
Background			
Age, mean $\pm$ SD	33.0 $\pm$ 4.7	31.1 $\pm$ 4.3	0.04*
Pre-pregnancy BMI, mean $\pm$ SD	24.2 $\pm$ 3.8	23.1 $\pm$ 2.8	NS*
Nulliparous, n (%)	15 (38.5%)	38 (52.8%)	< 0.0001#
University education, n (%)	29 (74.4%)	52 (75.4%)	NS#
Working/Studying, n (%)	38 (97.4%)	67 (95.7%)	0.0004#
Married/Cohabiting, n (%)	36 (94.7%)	71 (100%)	0.0001#
Delivery / Post-partum			
Sleep > 8 hours, n (%)	10 (25.6%)	17 (23.6%)	< 0.0001#
Mode of delivery, n (%)			
Vaginal delivery	27 (67.5%)	61 (84.7%)	NS#
Vacuum extraction	7 (17.5%)	3 (4.2%)	NS#
Planned CS	3 (7.5%)	4 (5.6%)	0.00001#
Emergency CS	3 (7.5%)	4 (5.6%)	0.00001#
Premature birth, n (%)	0 (0%)	1 (1.4%)	NS#
Gestational age at birth, mean weeks [min,max]	40.5 [38.4, 42.1]	40 [34.7, 42.3]	0.0208*
Sex of fetus, female n (%)	24 (60%)	33 (45.8%)	NS#
Breastfeeding, n (%)	34 (89.5%)	68 (95.8%)	0.006#

SD standard deviation. * Student's *t*-test. # Chi-square test. NS, not significant (p-value > 0.05).

3.1 DEGs in postpartum week 8 (ppw8) vs. gestational week 38 (gw38)

Healthy samples at gw38 and ppw8 showed an evident separation, indicative of differences in global transcriptional profiles. The two components explained 30% and 10.3% of the variance, PC1 and PC2, respectively (Figure 2).

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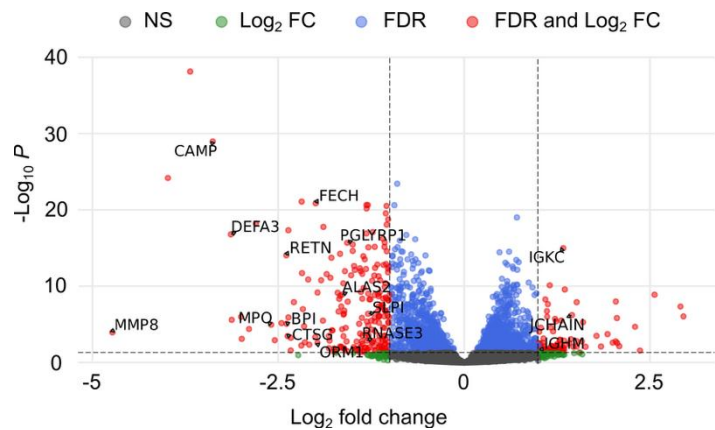
221

222 **Figure 2. Principal components (PC) of transcriptomic profiles during pregnancy and postpartum.**

223 Plot of the distribution of healthy samples between the timepoint of gestational week 38 (gw38) and postpartum
224 week 8 (ppw8) with minimal overlap between the two groups. Each point represents one sample (n = 112). Colors
225 indicate sampling timepoints. Ellipses represent 95% confidence regions. PC1 and PC2 explain 30% and 10.3 %
226 of the total variance, respectively.

227

228 A total of 293 DEGs were found when comparing the late pregnancy with the postpartum
229 transcriptome ($\log_2FC$: [-4.7,3]; Additional File 2 - Table S1). Postpartum, 210 of these DEGs
230 were downregulated, and 83 were upregulated (Figure 3).



231

232 **Figure 3. Differentially expressed genes between pregnancy and postpartum.**

Volcano plot of DEGs at postpartum week 8 vs. gestational week 38, displaying the statistical significance and log fold differences. The most significantly down-regulated DEGs included *MMP8*, *CAMP*, *DEFA3*. Legend: red dots, statistically significant genes above FC threshold ($|\log_2FC| > 1$ and $FDR < 0.05$); blue dots, statistically significant genes under FC threshold ($|\log_2FC| < 1$ and $FDR < 0.05$); green dots, not statistically significant genes above FC threshold ($|\log_2FC| > 1$ and $FDR > 0.05$); grey dots, not statistically significant genes under FC threshold ($|\log_2FC| < 1$ and $FDR > 0.05$).

The top five downregulated DEGs were *MMP8*, *LTF*, *LCN2*, *CAMP*, and *DEFA3*, which encode neutrophil-associated antimicrobial and inflammatory mediators ($\log_2FC$: [-4.7, -3.1]). Upregulated DEGs were predominantly enriched in adaptive immune processes, especially immunoglobulin-related genes, with *CACNG6*, *HDC*, and *SLC45A3* showing the highest levels of upregulation (top three $\log_2FC$: [2.56, 2.95]).

3.2 DEGs in within-subject analyses

The paired analysis of the transcriptome, adjusting for age and BMI, determined 45 downregulated and two upregulated genes from late pregnancy to postpartum ($\log_2FC$: [-8.1, 5.4], with 27 of them in common with the between-subject analysis (Additional File 1 - Figure S1, Additional File 2 - Table S1). The overlapping DEGs were regulated in the same direction and included *CAMP*, *MMP8*, *LTF*, *DEFA3*, *BPI*, and *FCER1A* ($\log_2FC$: [-7.8, 4]). Among these 27 common DEGs, *FCER1A* was the only gene with increased expression during the postpartum period.

3.3 Enrichment in Biological Processes (BP)

Functional enrichment analysis showed that 210 downregulated genes from the main cross-sectional model, comparing ppw8 to gw38, were significantly associated with 42 BP (Additional File 2 - Table S2, $FDR < 0.05$), linked to humoral immune response, erythrocyte differentiation, erythrocyte development, and defense response to bacterium (Figure 4A).

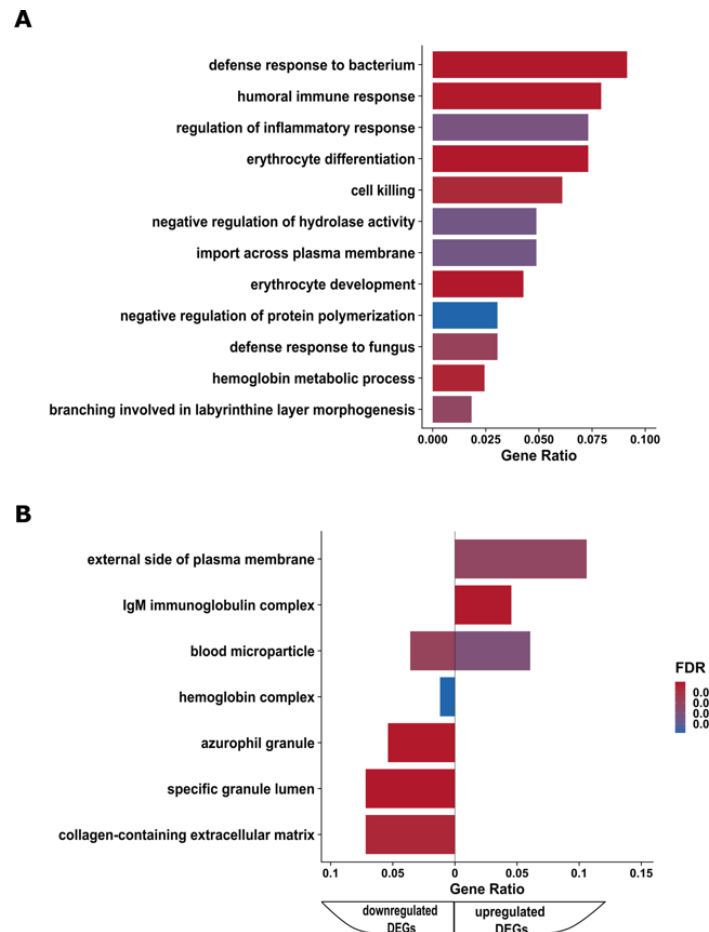
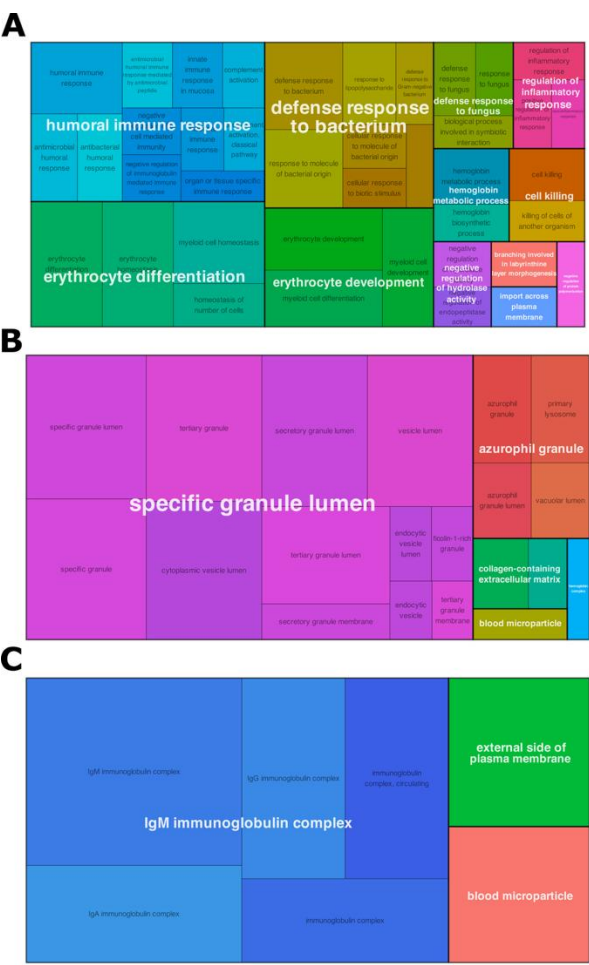


Figure 4. Biological Processes and Cellular Components enrichment of DEGs in the main model (ppw8 vs. gw38).

Processes in which DEGs are involved as shown by gene set overrepresentation. **A)** Reduced representation of enriched Biological Processes (BP) in downregulated DEGs. The enrichment among upregulated genes did not yield any pathways passing significance criteria (FDR < 0.05). **B)** Reduced representation of enriched Cellular Components (CC) for down- and up-regulated genes in ppw8 vs gw38. Bar length reflects the gene ratio (percentage of significant DEGs in the given ontology term). Color intensity represents adjusted p-values (FDR). Downregulated terms highlight erythropoiesis and immune defense pathways, while upregulated terms involve immunoglobulin complexes and the external side of plasma membrane.

Several processes related to immune response, such as response to lipopolysaccharide, antimicrobial and antibacterial humoral response, as well as processes related to the placenta, maternal-fetal exchange, and cellular transport, showed significant downregulation (Additional File 2 - Table S2, Figure 4A and 5A).



276 **Figure 5. Treemap of the top reduced GO terms between pregnancy and postpartum.**

277 **A)** Treemap of reduced GO Biological Processes (BP) terms of downregulated DEGs, **B)** Treemap of reduced GO
278 Cellular Components (CC) terms of downregulated DEGs. **C)** Treemap of reduced GO Cellular Components (CC)
279 terms of upregulated DEGs. The rectangle size is proportional to the enrichment significance: larger blocks
280 correspond to the most significant GO terms, grouping similar terms by semantic similarity.

282 In contrast, BP enriched in upregulated DEGs postpartum involved adaptive immune
283 pathways. However, these processes enriched for immunoglobulin-related genes did not
284 remain significant after multiple-testing adjustments.

285 Downregulated genes in postpartum women compared to women at gw38, that
286 were enriched in BP related to innate immune defense, included *LTF* (lactotransferrin), *CTSG*

(cathepsin G), *CAMP* (cathelicidin antimicrobial peptide), *DEFA3* (defensin alpha 3), *PGLYRP1* (peptidoglycan recognition protein 1), *BPI* (bactericidal permeability-increasing protein), *SLPI* (secretory leukocyte protease inhibitor), *MPO* (myeloperoxidase), *RNASE3* (eosinophil cationic ribonuclease), and *MMP8* (matrix metalloproteinase 8).

3.4 Enrichment in Cellular Components (CC)

CC enriched in downregulated DEGs, as a result of GOEA, were mostly related to specific granule-associated structures (Figure 4B). The secretory granule lumen, cytoplasmic vesicle lumen, and vesicle lumen had the highest gene ratios, followed by the specific granule and tertiary granule (Additional File 2 - Table S2). Other significant CC enriched in downregulated DEGs included collagen-containing extracellular matrix, azurophil granule, hemoglobin complex, and blood microparticle (Figure 5B).

Compartments enriched in upregulated DEGs reflected immunoglobulin-associated structures, the external side of the plasma membrane and blood microparticles (Figure 4B and Figure 5C), which were driven by immunoglobulin-associated genes, *IGHM* (immunoglobulin heavy constant mu), *IGKC* (immunoglobulin kappa constant), *JCHAIN* (joining chain of multimeric IgA and IgM), *FCER1A* (high-affinity IgE receptor subunit alpha), *IGLC2* (immunoglobulin lambda constant 2), and *FCER2* (low-affinity IgE receptor), upregulated in postpartum women compared to women at gw38. Further enrichment analyses did not reveal significant pathways in the KEGG database.

3.5 Deconvolution of transcriptomics data for cell-type specific analysis

Due to the heterogeneity of whole blood and the well-documented leukocyte shifts between pregnancy and postpartum states (40), we deconvoluted cell-type proportions from transcriptomic data. As shown in Additional File 1 - Figure S2, compared with gw38, women at ppw8 had higher abundance of memory B-cells, CD8⁺ cells, resting memory CD4⁺ cells, Tregs, activated NK cells, and resting dendritic cells ($p < 0.02$ for all) and lower activated dendritic cell and neutrophil count ($p = 0.002$ for monocytes and $p < 0.001$ for activated dendritic cells and neutrophils).

Given the significant changes in cell type composition, we repeated the DEG analysis, adjusting for BMI and age, as well as for cell type composition, gestational length and sex of the fetus between gw38 and ppw8. This analysis revealed 129 DEGs ($\log_2FC$: [-3.1, 1.4], Additional File 1 - Figure S2B, Additional File 2 - Table S1). Notably, among these DEGs, 101 DEGs overlapped with the ones indicated above when correcting only for age and BMI (Figure 6).

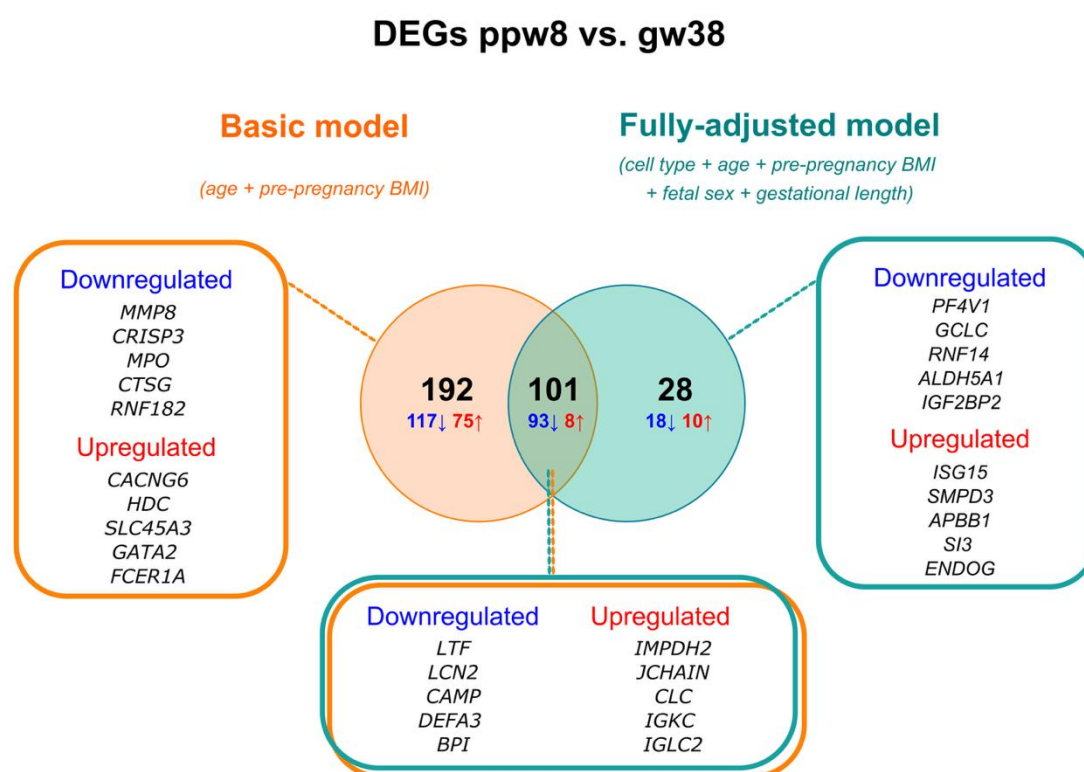


Figure 6. Venn diagram of DEGs in two cross-sectional models

Venn diagram showing the overlap of differentially expressed genes (DEGs) between two cross-sectional models. The orange circle represents the age- and BMI-adjusted model, whereas the teal circle represents the model additionally adjusted for cell-type composition, gestational age at birth, and fetal sex. Numbers indicate the total number of DEGs in each section. Within each sector, downregulated genes are shown in blue with a downward arrow, and upregulated genes are shown in red with an upward arrow. For each sector, five representative upregulated and five representative downregulated genes are displayed, selected from the top-ranked regulated genes.

These included down-regulated genes involved in innate immunity, such as *CAMP*, *LTF*, *DEFA3*, *BPI*, *PGLYRP1*, and upregulated immunoglobulin-related genes, such as *IGKC* and *JCHAIN* (Additional File 2 - Table S1). The 28 genes unique to this analysis are primarily involved in cellular metabolism, mitochondrial function, gene expression, and immune response (Figure 6).

The enrichment analysis of the 129 DEGs was conducted separately for downregulated and upregulated genes and yielded 52 GO terms and no significant KEGG pathways in total. Among enriched pathways across three ontologies, 46 were enriched among downregulated DEGs (27 BP, 10 CC and 9 MF; Additional File 2 - Table S3), and 38 were common to the model that only corrects for age and BMI (21 BP among downregulated, 9 CC among downregulated, 5 CC among upregulated, 2 MF among downregulated, and 1 MF among upregulated DEGs). The processes such as innate immunity, defense mechanisms and myeloid cell differentiation, as well as granule components, were also significant in the further adjusted model. The pathway enrichment of upregulated genes again involved immunoglobulin-related components. The unique GO terms were identified only in the enrichment of downregulated genes and involved intracellular, gene regulatory, and metabolic pathways such as apoptotic signaling, mRNA stabilization and the extrinsic component of the postsynaptic membrane (Additional File 2 - Table S3, Additional File 1 - Figure S3).

Considering the 101 genes in common between the two models (Figure 6), the direction of differential expression was perfectly conserved across the two cross-sectional analyses, with no common genes showing a sign change in $\log_2FC$, indicating that the inclusion of cell-type composition covariates did not invert the regulatory direction of any robustly differentially expressed gene. Quantitatively, the $\log_2FC$ estimates were strongly correlated between models (Spearman's $\rho = 0.75$, $p < 0.0001$; Additional File 1 - Figure S4). Linear regression of $\log_2FC$ for the fully-adjusted model on $\log_2FC$ for the main model yielded a slope of 0.90 (SE = 0.027, $p < 0.0001$), corresponding to an average attenuation of approximately

10% in effect-size magnitude, and an intercept of -0.12 ($p = 0.008$), reflecting a small systematic offset consistent with model rescaling after inclusion of additional covariates.

4. Discussion

This study investigated peripheral blood transcriptomic differences between late pregnancy (gw38) and the postpartum period (ppw8) among healthy women. We observed downregulation of genes involved in erythropoiesis and antimicrobial response, accompanied by upregulation of pathways related to adaptive immunity, but no significant changes in endocrine pathways in the postpartum period compared with late pregnancy. Within-subject analyses in a very small sub-sample, and analyses that accounted for additional covariates, replicated the findings for several transcripts. The non-overlapping transcripts highlighted processes related to metabolism, gene regulation, and mitochondrial function when accounting for cellular shift. Together, these findings suggest the dynamic nature of the maternal transcriptome, reflecting immune rebalancing, as it transitions from pregnancy toward a non-pregnant state. Further adjustment for cell-type composition, gestational length, and fetal sex revealed additional intracellular dynamics apart from immune mechanisms in the peripartum transcriptome.

From a functional perspective, the differences in gene expression indicated a downregulation of hematopoiesis and innate immunity from late pregnancy to postpartum period. Reduced expression of genes involved in erythrocyte development and homeostasis during postpartum likely reflects a physiological return of maternal circulation and hematologic characteristics to the non-pregnant state (11). Innate and antimicrobial immune responses were also downregulated, suggesting immune rebalancing following pregnancy-related adaptations in immune system (7). At the cellular (CC) level, the suppression of granules, lysosomes, and vesicle-associated lumens suggested diminished capacity for degranulation and antimicrobial activities (41). Such changes may transiently increase susceptibility to postpartum infections, such as endometritis and mastitis, and reactivation of latent infections

(6, 42, 43). Overall, the downregulated genes involved in innate immunity were consistently enriched across biological and cellular ontologies, mirroring a reversal of transcriptomic alterations observed throughout gestation (11).

The key downregulated genes, such as *LTF*, *CAMP*, *DEFA3*, and *BPI*, further supported a broad reduction in neutrophil- and granulocyte-mediated antibacterial activity. *CAMP* and *BPI* have been reported to be of fetal origin (44), which may explain their decline postpartum. Interestingly, these key downregulated genes have been shown to exhibit anti-inflammatory properties in addition to their inflammatory functions (45, 46). This finding aligns with reduced levels of postpartum anti-inflammatory proteins, as previously shown in the BASIC cohort (47). In a case-control transcriptomic study of perinatal depression, including healthy subjects from the current study, we previously observed upregulation of innate defense genes, suggesting altered immune physiology in postpartum depression (15).

Interestingly, immunoglobulin-related genes that are involved in adaptive immunity, such as *IGHM*, *IGKC*, *JCHAIN*, and *FCER1A*, were upregulated postpartum. During gestation, the maternal adaptive immune system is suppressed to maintain fetal tolerance (48, 49). According to our data, this suppression appears to be lifted postpartum, enabling the reactivation of adaptive immune functions. Increased immunoglobulin transcripts postpartum could indicate a return to heightened immune surveillance and antibody production as well as contribute to neonatal passive immunity through breastfeeding (47, 50). However, this immune reactivation may also carry risks, such as the potential for autoimmune flare-ups in women with predisposing conditions (51). Moreover, despite the substantial endocrine changes occurring throughout the peripartum period, our study did not reveal corresponding alterations in endocrine-related gene expression, such as a reduction in placental hormones as previously shown (10).

The observed immune-related transcriptomic differences align with existing knowledge that postpartum women undergo complex immunological shifts (52). While previous molecular studies have primarily focused on immune adaptations of pregnancy (7, 10), this

study points to a heightened adaptive immune state after childbirth (53). Collectively, these findings from different omics support that the postpartum period involves a system-wide immune reprogramming that may be protective, but if dysregulated, could also render postpartum women more susceptible to infection, contribute to postpartum autoimmune flare-ups, or affect maternal mental health through inflammatory signaling pathways (5, 6, 54).

Notably, large differences in both cell-type composition and mRNA expression profiles are expected between pregnancy and postpartum samples (10, 40). Whole blood is a heterogeneous tissue, which can lead to variation in leukocyte proportions reflected in transcriptomic differences (12). Thus, the findings from the only age- and BMI-adjusted model should be interpreted as reflecting broad systemic immune changes, accompanied by profound changes in granulocytes, erythroid cells, and lymphocytes. Importantly, the additional adjustment for cell-type composition and obstetric variables produced only modest attenuation of differential expression estimates, with highly consistent directionality across models. This supported the interpretation that the fully-adjusted model removed a very small fraction of variance attributable to compositional confounding, preserving the underlying biological signal observed in the main analysis.

Complementary to this, as expected, the cell-type-adjusted model yielded fewer DEGs and attenuated several leukocyte-related signals in pathway enrichment. However, a core subset of immune-related transcripts, including *CAMP*, *BPI*, *IGKC*, and *JCHAIN*, remained differentially expressed after adjustment, suggesting that these signals are not exclusively attributable to shifts in cell-type proportions. Together with the enrichment results, these findings point to a physiologically relevant changes in the maternal immune landscape during the peripartum transition or persistent postpartum remodeling alongside cell-intrinsic and gene-regulatory changes, reflecting immune rebalancing after pregnancy. Thus, the maternal transcriptome appears to transition from the gestational immune state toward postpartum immune remodeling through both cellular composition shifts and sustained transcriptional regulation. If validated, these signatures may help distinguish pregnancy and

postpartum states and provide insight into the molecular reorganization of the maternal immune system across the peripartum period.

The within-subject analysis, although limited by the number of paired samples, revealed a transcriptomic shift from late pregnancy to postpartum. Notably, DEGs identified in within-subject analysis, including *CAMP*, *MMP8*, *LTF*, *DEFA3*, and *BPI*, showed decreased expression, consistent with downregulation of these genes in the between-subject comparison, while *FCER1A* was consistently upregulated across both analyses. Despite the small sample size, this overlap is in line with the dual-pattern immunological transition during the peripartum period with downregulation of innate immunity and upregulation of adaptive immune responses. Though these findings should be interpreted cautiously due to the small sample size, the non-overlapping DEGs in the paired analyses revealed only additional down-regulated defense genes and upregulated pro-inflammatory genes, along with unique genes involved in signal transduction and stress response.

Our results from both within- and between-subject analyses complement those of two other studies by comparing healthy women with term births in late pregnancy (gw38) and postpartum (ppw8). Among the 39 DEGs reported by Pan *et al.* based on cell-free RNA collected from 60 women across three trimesters and within 24 hours after delivery (10), 10 (25.6%) were downregulated postpartum in the present study in both between-subject and paired analyses. Even though these 10 genes were associated with different temporal trajectories throughout pregnancy in their study, most of them were under-expressed in plasma postpartum (10). The downregulation of *MMP8/9*, *CAMP*, and *DEFA3* observed in the present study is complementary to prior findings from a Danish cohort of 14 women, which also display a decreased expression of these antibacterial genes three months postpartum. In contrast to Wright *et al.*, where most of these DEGs lost significance after cell type adjustment (12), the majority remained significant in our cell type-adjusted model, which might reflect differences in statistical power due to smaller longitudinal cohorts. Despite the differences in sampling times after delivery across these studies examining transcriptomics of late pregnancy and

postpartum, overlapping genes have emerged, especially those related to immune defense. Therefore, these results call for dense longitudinal sampling, including earlier and later postpartum time points, to further characterize the temporal dynamics of transcriptomic shifts of the peripartum period and to determine the association of these transcriptomic differences with health outcomes and disease risk.

This study has several important strengths, including the explorative approach on the whole transcriptome level to examine the complete set of protein-coding RNA transcripts, providing valuable insight into systemic gene expression patterns and reference knowledge to study transcriptomics of relevance to perinatal maternal health. The sample size of the present cross-sectional study overcomes the limitations of previous studies based on small samples. In addition, the integration of both paired and unpaired analyses added depth to our findings, as the paired comparisons provided preliminary within-subject results in line with the larger unpaired analyses, allowing detection of peripartum transcriptomic differences over time. Furthermore, potential covariates such as cell type composition, sex of the fetus, and gestational length were considered. Nonetheless, limitations should also be acknowledged. The sample size for paired analyses was modest. Furthermore, our sampling did not extend to earlier or later postpartum time points, limiting the ability to capture dynamic trajectories across the peripartum period.

5. Conclusions

Our findings identified several differentially expressed genes when comparing gestational week 38 to postpartum week 8, indicating changes in erythropoiesis, metabolism and immune response across the perinatal transition in healthy women. The postpartum period was accompanied by downregulation of innate immune and granulocyte-mediated antibacterial pathways, with reduced expression of key antimicrobial effector genes such as *LTF*, *CAMP* and *BPI*. In parallel, erythroid and myeloid differentiation pathways were also downregulated, reflecting a post-gestational shift in hematopoietic priorities. These immune and erythropoietic

patterns largely remained across longitudinal and cell-composition corrected analyses, whereas the adjustments for leukocytes also introduced cell-intrinsic pathways related to gene regulation and metabolism. These transcriptomic adaptations likely reflect physiological alterations in immune homeostasis following pregnancy, from innate to adaptive immune profile, providing a framework for further understanding of the postpartum period and its implications for maternal health.

6. List of abbreviations

BASIC: Biology, Affect, Stress, Imaging and Cognition cohort

BMI: Body mass index

BP: Biological processes

BPI: Bactericidal permeability-increasing protein

CACNG6: Calcium voltage-gated channel auxiliary subunit gamma 6

CAMP: Cathelicidin antimicrobial peptide

CC: Cellular components

CTSG: Cathepsin G

DEFA3: Defensin alpha 3

DEG: Differentially expressed genes

EPDS: Edinburgh Postnatal Depression Scale

FCER1A: High-affinity IgE receptor subunit alpha (Fc epsilon receptor Ia)

FCER2: Low-affinity IgE receptor (Fc epsilon receptor II)

FDR: False discovery rate

513	GO: Gene Ontology
514	GOEA: Gene Ontology Enrichment Analysis
515	gw38: Gestational week 38
516	<i>HDC</i> : Histidine decarboxylase
517	<i>IGHM</i> : Immunoglobulin heavy constant mu
518	<i>IGKC</i> : Immunoglobulin kappa constant
519	<i>IGLC2</i> : Immunoglobulin lambda constant 2
520	<i>JCHAIN</i> : Joining chain of multimeric IgA and IgM
521	KEGG: Kyoto Encyclopedia of Genes and Genomes
522	<i>LCN2</i> : Lipocalin 2
523	log ₂ FC: log ₂ -transformed fold change
524	<i>LTF</i> : Lactotransferrin
525	MF: Molecular functions
526	<i>MMP8</i> : Matrix metalloproteinase 8
527	<i>MPO</i> : Myeloperoxidase
528	mRNA: messenger ribonucleic acid
529	<i>PGLYRP1</i> : Peptidoglycan recognition protein
530	ppw8: Postpartum week 8
531	RNA: Ribonucleic acid
532	<i>RNASE3</i> : Eosinophil cationic ribonuclease
533	<i>SLC45A3</i> : Solute carrier family 45 member 3

534 *SLPI*: Secretory leukocyte protease inhibitor

535

536 7. Declarations

537 7.1 Ethics approval and consent to participate

538 Informed consent was obtained from all participants. This study was approved by the Uppsala
539 Regional Ethical Committee (Dnr 2009/171).

540 7.2 Consent for publication

541 Not applicable.

542 7.3 Availability of data and materials

543 The datasets analyzed in the current study are available in the Gene Expression Omnibus
544 repository under the GEO Accession code GSE290313 at <https://www.ncbi.nlm.nih.gov/geo/>.

545 Scripts used are made available via Zenodo and GitHub
546 <https://doi.org/10.5281/zenodo.20623474>.

547 7.4 Additional Files

548 ***Additional_File_1.pdf* (Supplementary Figures)**

549 **Figure S1. Venn diagram of DEGs in three analyses.**

550 Venn diagram showing the overlap of DEGs across three models: (A) age- and BMI-adjusted main model, (B) paired
551 analysis, and (C) model additionally adjusted for cell type composition, gestational age at birth, and fetal sex (fully-
552 adjusted model). Numbers indicate the number of DEGs in each set and their overlaps. Thirteen genes were
553 consistently identified across all three analyses, including *AHSP*, *BPI*, *CAMP*, *DEFA3*, *F5*, *LCN2*, *LTF*, *MCEMP1*,
554 *PGLYRP1*, *RETN*, *TENT5C* and *TLR5*. In each sector, the downregulated and upregulated genes were indicated
555 by blue and red color, respectively.

556 **Figure S2. Comparison of deconvoluted cell-types between gw38 vs. ppw8.**

557 Among 22 cell types deconvoluted using the model matrix, only 16 comparisons were performed due to the
558 presence of too many zeros in plasma cells, follicular helper T cells, gamma delta T cells, macrophages, activated
559 mast cells, and eosinophils. Women at ppw8 had higher abundance of memory B-cells, CD8+ cells, resting memory
560 CD4+ cells, Tregs, activated NK cells, and resting dendritic cells ($p < 0.02$ for all), whereas women at gw38 had

greater monocyte, activated dendritic cells, and neutrophil count ($p = 0.002$ for monocytes and $p < 0.001$ for activated dendritic cells and neutrophils).

Figure S3. Results of analyses upon inclusion of additional covariates along with cell-type principal components.

A) Principal component analysis (PCA) of transcriptomic profiles. **B)** Volcano plot of DEGs in sensitivity model. The most significantly down-regulated DEGs included *CAMP*, *DEFA3*, *BPI*. Legend: red dots, statistically significant genes above FC threshold ($|\log_2FC| > 1$ and $FDR < 0.05$); blue dots, statistically significant genes under FC threshold ($|\log_2FC| < 1$ and $FDR < 0.05$); green dots, not statistically significant genes above FC threshold ($|\log_2FC| > 1$ and $FDR > 0.05$); grey dots, not statistically significant genes under FC threshold ($|\log_2FC| < 1$ and $FDR > 0.05$). **C)** Reduced representation of enriched Biological Processes (BP) for downregulated genes and **D)** Cellular Components (CC) for down- and up-regulated genes in ppw8 vs gw38. Bar length reflects the gene ratio (percentage of significant DEGs in the given ontology term). Color intensity represents FDR-adjusted p-values. **E)** Treemap of reduced GO Biological Processes (BP) terms of downregulated DEGs, **F)** Treemap of reduced GO Cellular Components (CC) terms of downregulated DEGs.

Figure S4. Preservation of differential expression estimates after additional covariate adjustment.

Scatter plot comparing $\log_2FC$ estimates between the main age- and BMI-adjusted model and the fully adjusted model additionally including cell-type principal components, fetal sex, and gestational length. Each point represents a gene passing the significance criteria ($|\log_2FC| > 1$ and $FDR < 0.05$). The dashed diagonal line indicates identical $\log_2FC$ estimates between models. $\log_2FC$ direction was preserved for all 101 genes, common across two models, with no sign changes observed, and the estimates showed a strong positive correlation between models (Spearman's $\rho = 0.75$, $p = 1.74 \times 10^{-19}$).

Additional_File_2.xlsx (Supplementary Tables)

Table S1. DEGs for all the models (basic, fully-adjusted, and paired)

Table S2. GO Terms (BP, MF, CC) for basic model

Table S3. GO Terms (BP, MF, CC) for fully-adjusted model

7.5 Competing interests

The authors declare that they have no competing interests.

7.6 Funding

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7.7 Authors' contributions

EDP and MNM contributed equally as first authors. EDP, EA and SM conducted differential expression and pathway enrichment analyses and contributed to visualization. MNM and CZ analyzed demographic data and interpreted the transcriptomic findings. MNM, CZ and EC wrote the original draft and contributed to the visualization. EC, DdB, and AS conceptualized and designed the study, secured funding, and supervised. VB and FÖ critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

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