Supplemental Materials

1. Methods: Operationalization of variables

1.1 Psychological Distress Item Breakdowns

The Edinburgh Postnatal Depression Scale (EPDS; (1,2) consists of 10 items measured on a 4-point scale. Each item was anchored on a 0–3-point scale (0 - "*No, never*" and 3 - "*Yes, most of the time*").

The UCLA IRB requested that we drop the 10th item ("*The thought of harming myself has occurred to me"/ "He pensado en hacerme daño*"), due to the risks involved of asking about self-harm without offering follow-up psychiatric care. To honor this request, we use only the first 9 items of the EPDS scale, reproduced below in Table S1. Given the common concerns of the self-harm item in the EPDS, as stated in the manuscript, statistical analyses conducted by (3) show that the 10-item and 9-item EPDS are highly correlate with strong sensitivity.

Supplemental Table 1: Item Breakdown of EPDS Scale used in Wave 2 MCE studies

Reverse Coded	EPDS Scale Item Text	
	English	**Spanish**
	I have been able to laugh and see the funny side of things	He podido reír y ver el lado gracioso de las cosas
	I have looked forward with enjoyment to things	He mirado al futuro con placer para hacer cosas
x	I have blamed myself unnecessarily when things went wrong	Me he culpado sin necesidad cuando las cosas marchaban mal
	I have been anxious or worried for no good reason	He estado ansiosa y preocupada sin motivo
x	I have felt scared or panicky for no very good reason	He sentido miedo o pánico sin motivo alguno
x	Things have been getting on top of me	Las cosas me oprimen o agobian
x	I have been so unhappy that I have had difficulty sleeping	Me he sentido tan infeliz, que he tenido dificultad para dormir
x	I have felt sad or miserable	Me he sentido triste y desgraciada
x	I have been so unhappy that I have been crying	Me he sentido tan infeliz que he estado llorando

The Spielberger State-Trait Anxiety Inventory State scale (STAI; validated among pregnant women by (4), consists of six items, three of which are reversed coded, and anchored on a 4-point scale (1 = *Not at all* to 4 = *Very much*) with items like, "*I am worried*;"

Supplemental Table 2 Item Breakdown of STAI-SF Scale

Reverse Coded	STAI-SF Item Text	
	English	**Spanish**
x	I feel calm	Me siento calmada
	I feel upset	Me siento disgustada
x	I feel content	Me siento contenta
x	I am relaxed	Estoy relajada
	I am worried	Estoy preocupada
	I am tense	Estoy tensa

1.2. Mental Health Thresholds

The clinically significant cut-off thresholds presented in our paper were calculated the following ways.

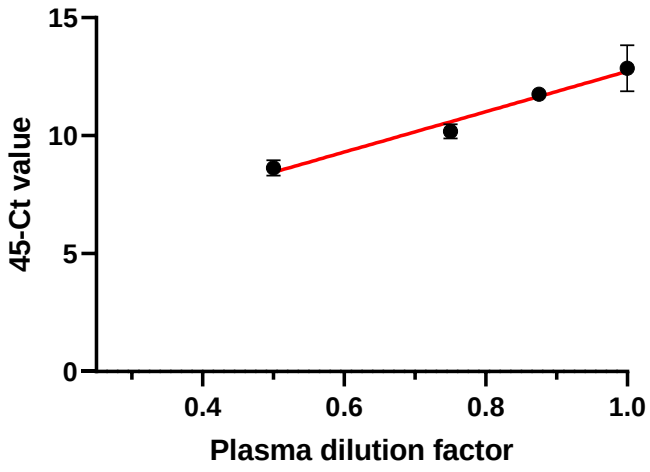
For the EPDS, scores were calculated based on a summation of individual items. These summary scores can usually range from 0-30 when all 10 items are included, and 0-27 when the self-harm question is dropped. Scores greater than 10 were identified as likely for at least minor depression. While there is some debate over the proper cut-off score to use, a validation of the EPDS among Spanish-speaking women suggests a cut-off threshold of 10 or 11 (5). Validation of the EPDS in a community sample of postnatal women against a diagnostic clinical interview found a positive predictive value of 73% of women scoring >10 (with 86% sensitivity) (2). By dropping the 10^{th} item of the EPDS, as required by the IRB, our cut-off of >10 is an even more conservative estimation of the number of women in our sample who may be experiencing symptoms of clinical depression.

For STAI-SF scale, scores were also based on a sum of individual items, with a range of 6-24. In the full-length scales, women range 20-80, and the broader literature often describes women with scores ≥40 as likely to have clinical levels of anxiety (6). We use a mean score with STAI-SF and extrapolate their methods to describe scores of 2 or greater as associated with clinical levels of anxiety (equivalent to a sum score of >12). The same cut-off has been used elsewhere (7). Since this dichotomization is only used for descriptive presentation of the cohort (e.g., Table 1 and Table S4-5).

1.3 Titration study on placental Extracellular Vesicle Assay

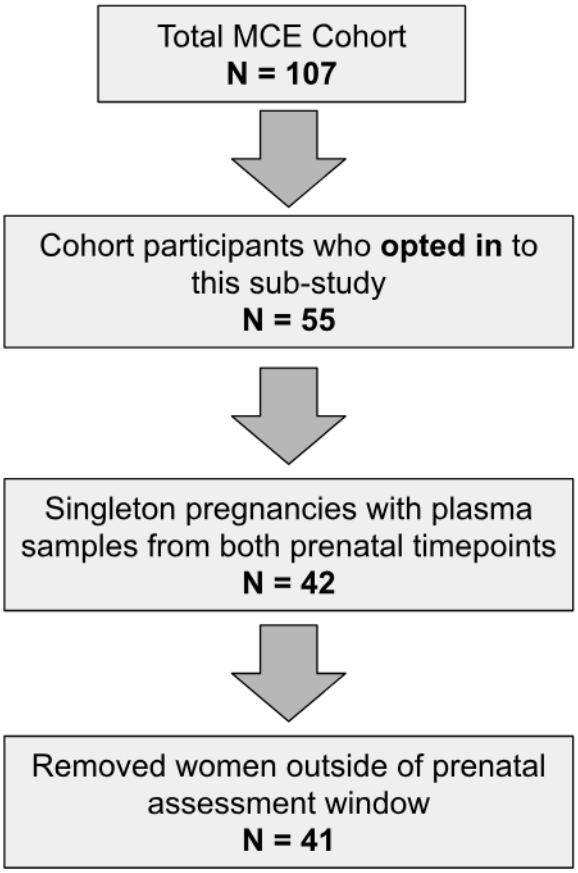
By conducting a dilution titration experiment with one sample of our 41 participants, run in duplicate we find a clear linear relationship between amount of plasma used and HLA-G+ pEV quantified by our methods. This supports the validity of the methods used.

Supplemental Figure 1: Plasma Titration Results



2. Results

Supplemental Figure 2: Selection of Analytic Cohort



2.1 Cohort Characteristics Split on Mental Health Cut-Offs

In Supplemental Table 4 and 5, we conducted t-tests and chi-squared tests to compare depressed versus non-depressed women and anxious versus non-anxious women in the cohort. The p-values (third column) reveal that there is no statistically significant difference between these groups in their general characteristics. There is a statistically significant difference between depressed and non-depressed women when it comes to HLA-G+ pEV quantity at Timepoint 2 and the ΔpEV quantity across gestation (Table S4), the remainder of the table variables are non-significant.

Supplemental Table 4: Depressed versus Not Depressed Women in Cohort

	Clinically Depressed (N=7)	Not Depressed (N=34)	P-value
Age (years)			
Mean (SD)	29.4 (5.28)	31.0 (5.54)	0.48
Median [Min, Max]	28.6 [21.7, 37.5]	30.3 [21.9, 41.8]	
Country of Origin			
U.S.	5 (71.4%)	14 (41.2%)	0.326

	Clinically Depressed (N=7)	Not Depressed (N=34)	P-value
Mexico	1 (14.3%)	13 (38.2%)	
Other Latin American Country	1 (14.3%)	7 (20.6%)	
Education			
Less than High School	1 (14.3%)	6 (17.6%)	NA
High School or Equivalent	6 (85.7%)	28 (82.4%)	
More than High School	0 (0%)	0 (0%)	
Relationship Status			
Yes	6 (85.7%)	31 (91.2%)	1
No	1 (14.3%)	2 (5.9%)	
Missing	0 (0%)	1 (2.9%)	
Food Insecurity			
Food insecure	1 (14.3%)	6 (17.6%)	1
Food secure	6 (85.7%)	28 (82.4%)	
Pre-Pregnancy Body Mass Index (BMI)			
Mean (SD)	29.3 (9.94)	30.3 (6.35)	0.841
Median [Min, Max]	25.8 [18.0, 41.8]	29.2 [20.2, 49.1]	
Missing	2 (28.6%)	3 (8.8%)	
Gravidity (Number of Times Pregnant)			
Mean (SD)	3.43 (2.99)	3.12 (2.50)	0.804
Median [Min, Max]	2.00 [1.00, 9.00]	3.00 [1.00, 14.0]	
Parity			
Nulliparous	3 (42.9%)	13 (38.2%)	1
Parous	4 (57.1%)	21 (61.8%)	
Anxiety at Timepoint 1			
Anxious	4 (57.1%)	11 (32.4%)	0.418
Not Anxious	3 (42.9%)	23 (67.6%)	
HLA-G+ pEV at Timepoint 1 (per 100 µL of plasma)			
Mean (SD)	12.8 (1.06)	12.6 (1.29)	0.666
Median [Min, Max]	12.7 [11.1, 14.2]	12.5 [9.90, 15.5]	
HLA-G+ pEV at Timepoint 2 (per 100 µL of plasma)			
Mean (SD)	11.2 (0.509)	12.1 (1.33)	0.00305
Median [Min, Max]	11.3 [10.3, 11.7]	11.9 [9.44, 15.9]	

	Clinically Depressed (N=7)	Not Depressed (N=34)	P-value
Change in HLA-G+ pEVs across pregnancy			
Mean (SD)	0.364 (0.215)	0.966 (0.827)	<0.001
Median [Min, Max]	0.272 [0.154, 0.730]	0.736 [0.160, 3.73]	
Gestational Age at Timepoint 1			
Mean (SD)	11.9 (4.50)	12.5 (2.27)	0.725
Median [Min, Max]	13.9 [5.57, 17.6]	12.6 [7.86, 17.0]	
Gestational Age at Timepoint 2			
Mean (SD)	25.0 (1.68)	25.3 (3.23)	0.788
Median [Min, Max]	25.3 [22.3, 27.3]	24.6 [20.6, 33.1]	
Smoker			
Yes	0 (0%)	8 (23.5%)	0.364
No	7 (100%)	26 (76.5%)	
Pre-eclampsia?			
Yes	1 (14.3%)	3 (8.8%)	1
No	6 (85.7%)	29 (85.3%)	
Missing	0 (0%)	2 (5.9%)	
Gestational Diabetes?			
Yes	1 (14.3%)	8 (23.5%)	0.909
No	6 (85.7%)	24 (70.6%)	
Missing	0 (0%)	2 (5.9%)	

Supplemental Table 5: Anxious versus Not Anxious Women in Cohort

	Anxious (N=15)	Not Anxious (N=26)	P-value
Age (years)			
Mean (SD)	31.2 (5.65)	30.5 (5.46)	0.716
Median [Min, Max]	29.6 [23.5, 41.8]	30.2 [21.7, 41.4]	
Country of Origin			
U.S.	5 (33.3%)	14 (53.8%)	0.144
Mexico	8 (53.3%)	6 (23.1%)	
Other Latin American Country	2 (13.3%)	6 (23.1%)	
Education			

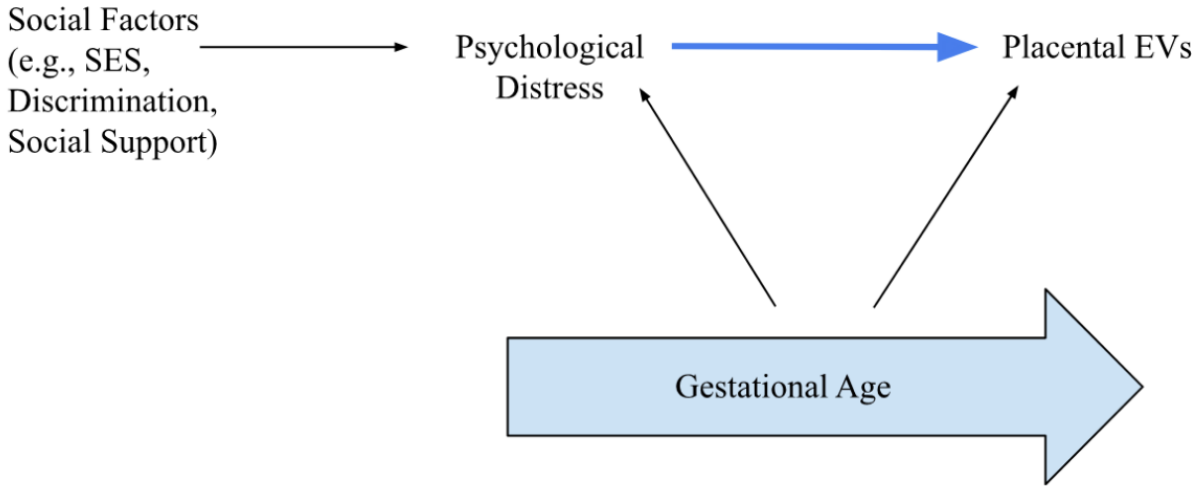
	Anxious (N=15)	Not Anxious (N=26)	P-value
Less than High School	3 (20.0%)	4 (15.4%)	NA
High School or Equivalent	12 (80.0%)	22 (84.6%)	
More than High School	0 (0%)	0 (0%)	
Relationship Status			
Yes	13 (86.7%)	24 (92.3%)	0.642
No	2 (13.3%)	1 (3.8%)	
Missing	0 (0%)	1 (3.8%)	
Food Insecurity			
Food insecure	3 (20.0%)	4 (15.4%)	1
Food secure	12 (80.0%)	22 (84.6%)	
Pre-Pregnancy Body Mass Index (BMI)			
Mean (SD)	28.6 (5.96)	31.0 (7.18)	0.29
Median [Min, Max]	29.1 [18.0, 41.8]	30.4 [20.2, 49.1]	
Missing	2 (13.3%)	3 (11.5%)	
Gravidity (Number of Times Pregnant)			
Mean (SD)	4.13 (3.50)	2.62 (1.63)	0.131
Median [Min, Max]	4.00 [1.00, 14.0]	2.00 [1.00, 6.00]	
Parity			
Nulliparous	6 (40.0%)	10 (38.5%)	1
Parous	9 (60.0%)	16 (61.5%)	
Clinical Depression (EDPS Sum >10) at Timepoint 1			
Clinically Depressed	4 (26.7%)	3 (11.5%)	0.418
Not Depressed	11 (73.3%)	23 (88.5%)	
HLA-G+ pEV at Timepoint 1 (per 100 µL of plasma)			
Mean (SD)	12.7 (1.57)	12.6 (1.04)	0.775
Median [Min, Max]	12.3 [9.90, 15.5]	12.6 [9.93, 14.2]	
HLA-G+ pEV at Timepoint 2 (per 100 µL of plasma)			
Mean (SD)	11.7 (1.07)	12.1 (1.37)	0.23
Median [Min, Max]	11.6 [9.44, 13.8]	11.9 [10.0, 15.9]	
Change in HLA-G+ pEVs across pregnancy			
Mean (SD)	0.695 (0.787)	0.960 (0.790)	0.308
Median [Min, Max]	0.541 [0.160, 3.31]	0.736 [0.154, 3.73]	

	Anxious (N=15)	Not Anxious (N=26)	P-value
Gestational Age at Timepoint 1			
Mean (SD)	11.9 (2.47)	12.7 (2.85)	0.331
Median [Min, Max]	12.3 [5.57, 15.4]	12.9 [6.00, 17.6]	
Gestational Age at Timepoint 2			
Mean (SD)	24.3 (3.19)	25.8 (2.81)	0.137
Median [Min, Max]	24.0 [20.6, 33.1]	25.6 [21.0, 31.9]	
Smoker			
Yes	3 (20.0%)	5 (19.2%)	1
No	12 (80.0%)	21 (80.8%)	
Pre-eclampsia?			
Yes	2 (13.3%)	2 (7.7%)	0.852
No	11 (73.3%)	24 (92.3%)	
Missing	2 (13.3%)	0 (0%)	
Gestational Diabetes?			
Yes	2 (13.3%)	7 (26.9%)	0.687
No	11 (73.3%)	19 (73.1%)	
Missing	2 (13.3%)	0 (0%)	

Timepoint 1 is the first prenatal assessment occurring at recruitment. Timepoint 2 is the second prenatal assessment occurring at their follow-up appointment (22-28 weeks gestational age).

2.2 Gestational Age

Supplemental Figure 3: Directed acyclic graph showing the proposed relationships between the variables of interest and potential confounders.



2.3 Operator and Batch Effects

Quantification with the EV-subpopulation assay at the UCLA Liquid Biopsy lab was conducted by two operators. The first two batches (or first two dates) were conducted with one operator and the remaining 10 batches were conducted with the second operator. Batch and operator effects are expected confounds for this manner of laboratory work. As described in the manuscript, the significant findings of Model 1 are replicated in Model 2. Model 2's design uses ΔpEV quantity across gestation. Since both participant timepoints are always measured on the same day by the same operator, operator and batch confounding should be inherently controlled for given the variable construction.

To be cautious, we still conducted t-test to compare the differences HLA-G+ pEV quantity between the two operators. We find that HLA-G+ pEV quantity was just significantly different between the two operators at timepoint 1 (t = -2.36, p-value = 0.05) and not statistically significant at timepoint 2 (t = -1.72, p-value = 0.14) or the change across pregnancy measure (t = -1.50, p-value = 0.15). Since our main findings were not significant for the cross-sectional analysis at timepoint 1, we do not further interrogate this issue.

For batch effects, we conducted two ANOVA tests exploring the relationship of HLA-G+ pEV quantity with date (a 12-level factor, where each date is a distinct batch). We find no statistical relationship between date and Ct-value at Timepoint 1 (Sum-Squares= 20.45, F-value = 1.30, p-value=0.27). For date and Ct-value at Timepoint 2, the ANOVA test revealed a relationship between date and Ct-value (Sum-squares= 34.97, F-value= 3.05, p-value= 0.01). We also find no statistically significant relationship between date and the ΔpEV across pregnancy measure (Sum-squares= 5.033, F-value= 0.667, p-value= 0.76). Since the batch effect is significant for timepoint 2, and we do find a significant effect in our main research question at timepoint 2, we interrogate this further to make sure our main finding is not an artifact of batch effects. We added date as a control variable to our significant main finding (Model 1b). For Model 1b anxiety remained significant above and beyond the effect of date (anxiety *ß*= -0.13, robust SE= 0.04, p-value= 0.002), while depression was just out of significance range, the betas remained with the same directions (depression *ß*=-0.05, robust SE=0.03, p-value = 0.081). The addition of a factor variable may also render the effect non-significant because of the power of the model given the low sample size. Operator and batch effects are not likely causing spurious results.

2.4 Possible Confounders

After running the models presented in the manuscript, it was suggested to us that our findings could be an artifact of increasing volume of blood during pregnancy. We already control for gestational age, which should take out any such effects. To explore this potential confound further, we evaluated the effect of pre-pregnancy body mass index (BMI) in the models. BMI is a known factor for influencing blood volume (8).

The addition of pre-pregnancy BMI rendered the previously significant Model 1b insignificant (depression *ß*=-0.071, robust SE= 0.04, p-value= 0.13; anxiety *ß*=-0.072, robust SE= 0.04, p-value= 0.11). For Model 2, depression remained significant above the effect of pre-pregnancy BMI (*ß*=-0.045, robust SE= 0.022, p-value= 0.047), and anxiety remained insignificant. Again, all the results maintained the same beta-value directions (negative), suggesting that the relationship between early-gestation psychological distress and later-gestation placental EV concentration is

likely a true finding but we are not powered to detect it with too many control variables. BMI may be a confounder that should be considered in future studies with larger sample sizes.

We also independently checked the effect of pre-eclampsia and gestational diabetes (GD) as confounders, since these are pregnancy complications associated with altered pEV concentrations. T-tests revealed no difference of HLA-G+ pEVs between pre-eclamptic and non-pre-eclamptic women as well as between GD and non-GD women for any pEV outcome variable.

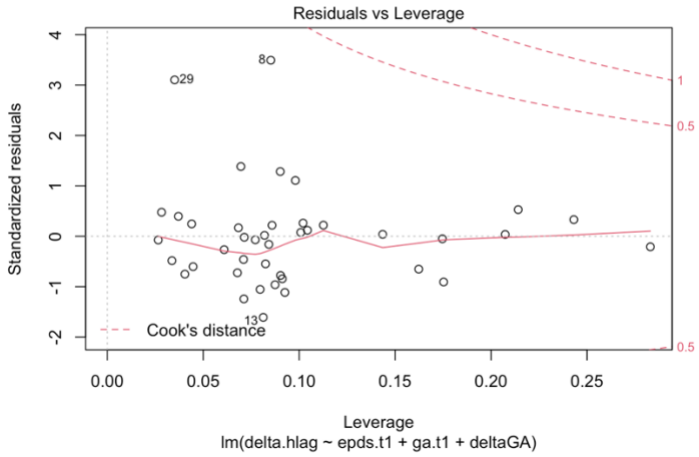
2.5 HLA-G+ Across Pregnancy

In Figure 2, we show the relationship of gestational age and HLA-G+ pEVs pooling all sub

2.5 Regression Diagnostics

In Models 1 and 2, no strong leverage points are beyond the Cook's distance (Figure S4).

Supplemental Figure 4: Exploration of Residuals and High Leverage Points



Variance Inflation Factors

Variance-inflation factors (VIF) were calculated to examine correlations between independent variables to quantify multicollinearity. While some scholars suggest a VIF of 5 or 10 to be problematic (9), others suggest a more conservative 2.5 limit (10). All VIF scores for all variables in all models were below 2.0, indicating no issues of multicollinearity.

Breusch-Pagan tests for Heteroskedasticity

For all models, we conducted Breusch-Pagan tests, which tests the null hypothesis of homoskedasticity. We failed to reject the null hypothesis only the Model 1b depression model. However, we used robust standard errors in all models to account for heteroskedasticity where present and remain conservative in our estimates throughout.

References

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