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Supplementary Material

Article Title: Longitudinal Changes in Sleep, Biological Rhythms and Light Exposure From Late Pregnancy to Postpartum and Their Impact on Peripartum Mood and Anxiety

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List of Supplementary Material for the article

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Appendix 1

Participant Recruitment

Prior to initiating the study, we performed a power calculation from a pilot study from our group, where 13 women with mood disorders and 17 matched controls completed clinical and actigraphy assessments during the third trimester of pregnancy and again between 6-12 weeks postpartum, which showed that we need 43 women to test for group differences with 80% power and an alpha level of 0.05. We aimed to enroll 50 women per group, in order to allow for 15% of attrition, resulting in a recruitment target of 100 women.

Study Procedures

At study enrollment, we collected the following information using a case report form: current medication, expected due date, current smoking status, age, family mood history, years of education, height and pre-pregnancy weight, gestational week, parity, whether the participant was a shift worker or had sleep apnea. During 1-3 and 6-12 weeks postpartum, we collected the following information: breastfeeding status, delivery date and method, changes in medication, and postpartum week.

Urinary 6-sulfatoxymelatonin

Following sample collection, participants refrigerated the sample until the sample pickup or drop-off during the morning of sample collection.

Creatinine levels in urine were analyzed by the Hamilton Regional Laboratory Medicine Program at St Joseph's Healthcare Hamilton (license no. 4037) using the Jaffe method (kinetic alkaline picrate; Abbott Diagnostics, Santa Clara, CA, USA).

Dim Light Melatonin Onset

During each visit, 2 mL of saliva was collected by participants once every hour from 6pm until midnight or bedtime, using the passive drool method into 15 mL presterilized conical centrifuge tubes (VWR, Radnor, PA). Once collected, samples were frozen by participants until sample pickup. Participants were instructed to fast from 5:00 pm onward on the night of sampling, and to rinse their mouth with water 10 minutes before collecting each sample. Participants were instructed to avoid 1) brushing or flossing their teeth, 2) to maintain a dimly lit environment during saliva collection and avoid screen exposure (e.g. television and smartphones), 3) to avoid naps on the day of saliva collection, 5) avoid drinking any fluids, chewing gum or smoking, 6) to avoid consuming alcohol within 12 hours of collection, 7) to avoid acidic or high sugar foods on the day of collection.

A total of 83 participants completed salivary sampling during the third trimester of pregnancy, 72 completed participants at 1-3 weeks postpartum, and 71 at 6-12 weeks postpartum. However, we were only able to obtain complete samples throughout all 3 visits that could be used to calculate dim light melatonin onset (DLMO) for a total of 12 participants.

Actigraphy

Data were collected in 1-minute epochs throughout the duration of monitor wear, and were extracted in the lab from the monitors using Philips Actiware (v.6.0). Sleep variables were extracted, and raw epoch-by-epoch data were exported to individual csv files from Actiware for biological rhythms variable extraction.

Cosinor Analysis
In cosinor analysis, time-series data are fitted to a cosine wave, using a regressive model. A single peak was extracted from a periodogram for each of the participants' circadian activity rhythms, approximating the endogenous period.

Non-parametric Circadian Activity Rhythm Analysis
Non-parametric circadian activity rhythm analysis permits the description of the rest-activity rhythm without the assumption that the rhythm follows a sinusoidal wave pattern – the classic, parametric way of rhythm analysis used by cosinor. Intradaily variability (IV) describes fragmentation of the activity rhythm, where large hourly differences in activity increase the value of the metric. For instance, daytime naps and nocturnal activity would increase values of IV. The typical range of this metric is 0-2[1].

Interdaily stability (IS), in turn, a metric that reflects synchronization of the activity rhythm to the external environment, including the light-dark cycle. IS reflects the variance of an average daily profile, divided by a total of the variance that is found across the entire data file[1].

The five least active consecutive hours of the day (L5) reflect the extent of activity during the rest phase. In turn, daytime activity is reflected by M10, a measure of the 10 most active consecutive hours of the day. The start times of these variables describe the timing of the least and most active periods of the day. Another metric that is calculated from these variables is relative amplitude, RA, which describes the amplitude of the rhythm, and is calculated as a ratio of the difference between the M10 and L5 measures, divided by a sum of M10 and L5[1].

Transition Probabilities
In brief, a two-state Hidden Markov Model was built for rest and active states, based on the idea that an observed time series is the product of an unobserved hidden state variable. The parameters of the Hidden Markov Model were calculated using the Baum-Welch algorithm.

Light Exposure:
Some outliers were found and removed for abnormally high light exposure (i.e. >200,000 lux exposure per hour, throughout 24 hours; third trimester n=4, 1-3 weeks n=1, 6-12 weeks postpartum n=5). MLiT500 and MLiT1000 values were unavailable for 1 participant, due to the low number of minutes exceeding these light thresholds.

Statistical Analysis
Normality of continuous variable distributions was tested using the Shapiro-Wilk test.

Generalized Estimating Equations (GEE)

Generalized Estimating Equations (GEE) are used to estimate generalized linear model parameters when observations are not thought to be independent – meaning that correlations are expected among variables. In the case of a longitudinal study design, it is likely that observations of predictors (sleep and biological rhythms variables) and outcomes (mood and anxiety) over time are related to each other. For instance, mood at 6-12 weeks postpartum is likely correlated to their mood at 1-3 weeks postpartum. Conversely, generalized linear models assume that observations are independent. GEE is based on fewer assumptions, and are semi-parametric. They are used to estimate the population averaged effects. GEE estimates the population average effect, and attempts to get robust estimates of the beta coefficients, that are less influenced by the correlation structure of the outcomes.

Generalized Estimating Equations (GEE) were used to model depression (EPDS) and anxiety (GAD-7) symptoms, using sleep/biological rhythms variables as predictors. GEE models are semi-parametric, and are used to estimate the population averaged effects. Quasi-likelihood independence model criterion (QIC, QICu)[2] was used to assess model fit (lower QIC values indicate better model fit). In instances of missing data from actigraph malfunction, 5-nearest-neighbour imputation was performed for predictor variables for visits with a filled-out EPDS. Visits for which EPDS scores were not available were not imputed.

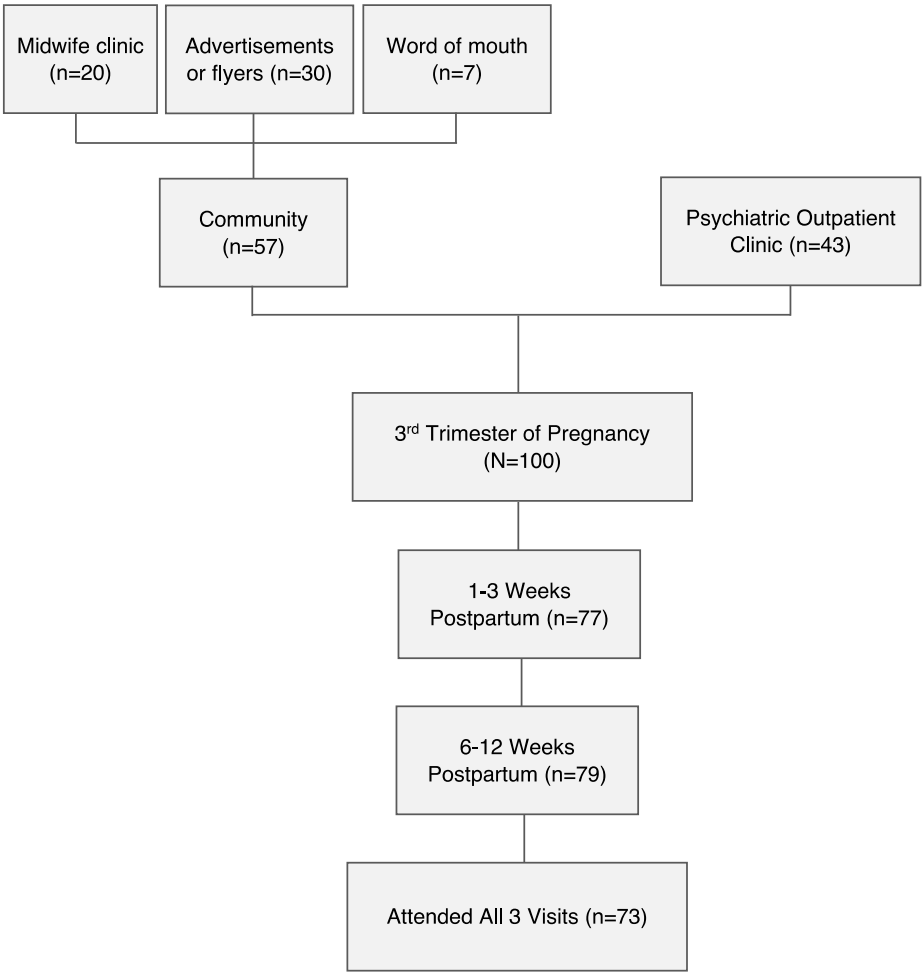
Next, we selected variables which had $p<0.01$ as predictors or had a significant variable by visit interaction as a predictor of depressive or anxiety symptoms in individual models. These were then used as predictors in final GEE models, where EPDS and GAD-7 were the primary outcomes. Additional predictors in these models consisted of age, years of education, BMI, history of mood or anxiety disorders, visit and the interaction of the sleep and biological rhythms variables with visit.

The statsmodels module (version 0.12) in Python 3.6 was used to estimate the GEE models (identity link function, autoregressive AR-1 working covariance structure). Other covariance structures were estimated, and autoregressive structure had the best fit.

References:

1. van Someren EJ, Hagebeuk EE, Lijzenga C, et al. Circadian rest—activity rhythm disturbances in Alzheimer's disease. Biological psychiatry 1996;40(4):259-270.
2. Pan W. Akaike's information criterion in generalized estimating equations. Biometrics 2001 Mar;57(1):120-125.

Supplementary Figure 1: Participant Flowchart



Supplementary Table 1: Longitudinal Changes in Dim Light Melatonin Onset					
Variable	**Third Trimester** **Mean±SD**	**1-3 Weeks Postpartum** **Mean±SD**	**6-12 Weeks Postpartum** **Mean±SD**	**Test**	**P value**
Dim Light Melatonin Onset (N=12)	20.77±1.08	20.63±0.73	20.84±1.27	F=0.11	0.89

Supplementary Table 2: Longitudinal Changes in Sleep and Rhythm Variables (n=57)								
Variable	**Third Trimester** **Mean±SD Median [IQR]**	**1-3 Weeks Postpartum** **Mean±SD Median [IQR]**	**6-12 Weeks Postpartum** **Mean±SD Median [IQR]**	**Test**	**P value (FDR Adjusted)**	***P value: Multiple Comparisons (FDR Adjusted)***		
						Third Trimester vs. 1-3 Weeks Postpartum	**Third Trimester vs. 6-12 Weeks Postpartum**	**1-3 Weeks vs. 6-12 weeks Postpartum**
MELATONIN METABOLITE								
6-SM	31.10 [24.72]	32.38 [19.92]	27.57 [28.60]	χ^2=1.22	0.47			
ACTIGRAPHY								
SLEEP VARIABLES								
TST	7.37 [1.00]	6.90 [1.34]	7.17 [1.02]	χ^2=2.15	0.12			
Sleep Onset Latency	12.14 [7.29]	10.29 [6.10]	10.86 [6.87]	χ^2= 1.50	0.35			
SE	**84.05 [5.02]**	**80.05 [4.20]**	**83.53 [3.38]**	**χ^2=5.43**	**0.00000073**	**0.00016**	0.78	**0.0000013**
WASO*	**62.75±20.37**	**85.40±22.25**	**64.91±18.99**	**F=28.73**	**0.00000000057**	**0.00000032**	0.65	**0.000000026**
Mean mid sleep time	27.37 [1.39]	27.33 [1.54]	27.42 [0.89]	χ^2=0.47	0.89			
Awakenings*	**28.12±8.55**	**23.02±5.34**	**23.44±6.31**	**F=23.46**	**0.000000018**	**0.0000047**	**0.0000072**	0.83
COSINOR VARIABLES								
Mesor*	**194.63±49.24**	**200.93±50.08**	**223.17±62.87**	**F=15.13**	**0.000027**	0.32	**0.00023**	**0.000078**
Amplitude	**146.55 [62.18]**	**147.37 [49.56]**	**178.22 [65.95]**	**χ^2=4.59**	**0.000051**	0.77	**0.0041**	**0.000081**
Acrophase	0.74 [0.36]	0.71 [0.35]	0.71 [0.34]	χ^2=1.40	0.39			
CQ	**0.78 [0.16]**	**0.77 [0.12]**	**0.84 [0.13]**	**χ^2=4.31**	**0.00013**	0.051	0.45	**0.00024**
Period	23.94 [0.28]	23.99 [0.20]	23.94 [0.32]	χ^2=1.73	0.25			
NON-PARAMETRIC CIRCADIAN ACTIVITY RHYTHM ANALYSIS								
IS	**0.58 [0.13]**	**0.52 [0.13]**	**0.58 [0.16]**	**χ^2=3.00**	**0.017**	**0.025**	1.00	0.062
IV*	**0.78±0.13**	**0.89±0.15**	**0.76±0.13**	**F=19.00**	**0.00000037**	**0.000017**	0.49	**0.0000017**
RA	**0.88 [0.08]**	**0.75 [0.10]**	**0.87 [0.13]**	**χ^2=7.23**	**0.000000000012**	**0.000000000056**	0.93	**0.0000000027**
L5	**17.14 [12.04]**	**44.81 [20.56]**	**26.73 [22.40]**	**F=7.84**	**0.00000000050**	**0.00000000000042**	**0.011**	**0.00024**
L5 Start time (n=48)	24.77 [1.21]	24.52 [2.16]	24.60 [1.61]	χ^2=0.52	0.90			

M10*	**303.64±78.08**	**313.76±78.27**	**354.79±102.54**	**F=17.94**	**0.00000075**	0.33	**0.000040**	**0.00010**
M10 Start time (n=49)	9.88 [2.33]	9.73 [1.82]	9.33 [2.00]	χ^2=1.48	0.36			
Nighttime Activity Mean*	**8800.68 ± 3835.39**	**17395.83 ± 6646.48**	**11280.49 ± 5158.74**	**F=55.41**	**0.00000000000000088**	**0.00000000000037**	**0.0076**	**0.000000013**
Nighttime Activity SD*	**5134.26 ± 4634.81**	**11939.40 ± 9199.01**	**6561.43 ± 5255.53**	**F=40.33**	**0.0000000000015**	**0.000000000053**	**0.035**	**0.00000022**
TRANSITION PROBABILITY ANALYSIS VARIABLES								
pAR day	0.05 [0.01]	0.04 [0.02]	0.04 [0.01]	χ^2=2.53	0.054			
pAR night	**0.10 ± 0.02**	**0.08±0.02**	**0.09±0.02**	**F=18.62**	**0.0000017**	**0.00000087**	**0.15**	**0.00015**
μ_A day	**410.43 [124.75]**	**394.55 [162.91]**	**443.78 [164.61]**	**χ^2=3.28**	**0.0068**	0.21	0.66	**0.011**
μ_A night*	**54.41±25.98**	**89.71±29.79**	**73.26±39.75**	**F=37.76**	**0.00000000050**	**0.000000000046**	**0.0036**	**0.00015**
pRA day	**0.06 [0.01]**	**0.08 [0.03]**	**0.08 [0.01]**	**χ^2=7.12**	**0.000000000018**	**0.000000000059**	**0.00016**	0.050
pRA night	**0.10 [0.03]**	**0.08 [0.03]**	**0.09 [0.03]**	**χ^2= 4.87**	**0.000012**	**0.000021**	**0.000063**	1.00
μ_R day	**69.76 [52.60]**	**34.81 [32.85[**	**45.35 [51.77]**	**χ^2=5.58**	**0.00000034**	**0.00000062**	0.093	**0.016**
μ_R night	**0.00 [0.04]**	**0.00 [0.00]**	**0.00 [0.00]**	**χ^2=2.99**	**0.016**	**0.025**	**0.031**	1.00
LIGHT EXPOSURE VARIABLES								
MLiT10 (n=49)	14.00 [0.94]	14.22 [0.90]	14.19 [0.66]	χ^2=0.51	0.89			
MLiT100 (n=49)	13.62 ± 0.93	14.07±0.89	13.65±1.14	F=3.61	0.064			
MLiT500 (n=48)	13.70±1.25	14.10±1.19	13.57±1.66	F=2.17	0.17			
MLiT1000 (n=48)	**13.71 [1.80]**	**14.25 [1.78]**	**13.43 [1.68]**	**χ^2=2.76**	**0.032**	0.32	0.82	**0.046**
TAT10 (n=49)	505.00 [215.31]	431.79 [279.01]	416.33 [273.77]	χ^2=1.31	0.42			
TAT100 (n=49)	**164.21 [149.08]**	**103.57 [102.44]**	**128.21 [150.85]**	**χ^2=2.63**	**0.044**	0.062	0.77	0.44
TAT500 (n=49)	56.77 [91.90]	40.62 [60.95]	51.69 [101.38]	χ^2=1.72	0.25			
TAT1000 (n=49)	32.38 [70.62]	28.85 [56.57]	41.23 [80.07]	χ^2=1.82	0.21			

* log transformed; Bolded text: survived FDR Correction

Abbreviations: 6-SM – 6-sulfatoxymelatonin; CQ - Circadian Quotient; L5 -5 consecutive lowest-activity hours; M10 - 10 consecutive hours with highest activity; MLiT - Mean timing of light exposure; pAR - probability of transitioning from active to rest state; pRA - probability of transitioning from rest to active state; SD - Standard Deviation; SE - Sleep Efficiency; TAT - Time Above Light Threshold; TST - Total Sleep Time; WASO - Wake After Sleep Onset; μ_A mean activity during active state; μ_R mean activity during rest state.

Supplementary Table 3: Using Generalized Estimating Equations to Model Depressive Symptoms (EPDS) as Function of the Most Predictive Sleep and Biological Rhythms Variables Across Perinatal Period (n=72)

Variable	ß	Std Err	P Value
Intercept	**-19.65**	**6.42**	**0.0022**
Visit 2 (1-3 Weeks Postpartum)#	13.84	8.59	0.11
Visit 3 (6-12 Weeks Postpartum)#	**19.08**	**8.13**	**0.019**
Age	0.031	0.077	0.69
Years of Education	0.096	0.13	0.46
BMI	0.038	0.046	0.40
Past History of Mood Disorders (Yes)	0.67	0.73	0.35
Past or Current Anxiety Disorder (Yes)	**2.44**	**0.72**	**0.00073**
BRIAN	**0.21**	**0.043**	**0.0000010**
Visit 2:BRIAN	-0.0067	0.047	0.89
Visit 3:BRIAN	-0.0356	0.041	0.39
μ_R night	**23.27**	**6.54**	**0.00037**
Visit 2: μ_R night	**-22.82**	**6.93**	**0.0010**
Visit 3: μ_R night	**-26.46**	**7.14**	**0.00021**
pRA Night	17.01	14.87	0.25
Visit 2: pRA Night	-30.40	19.45	0.12
Visit 3: pRA Night	**-50.59**	**20.45**	**0.013**
CQ	**6.41**	**2.91**	**0.027**
Visit 2: CQ	**-9.83**	**4.76**	**0.039**
Visit 3: CQ	-2.88	3.25	0.38
IV	-1.98	2.38	0.41
Visit 2:IV	6.23	4.22	0.14
Visit 3:IV	-0.81	3.23	0.80
μ_A night	0.021	0.016	0.19
Visit 2: μ_A night	-0.016	0.021	0.45
Visit 3: μ_A night	-0.011	0.018	0.54
TST	0.81	0.50	0.11

Visit 2: TST	-0.89	0.64	0.16
Visit 3: TST	-1.26	0.82	0.12

Model QIC: 699.08; **QICu** = 706.15. #Reference level: Third Trimester of Pregnancy. **Abbreviations:** BMI – Body Mass Index; BRIAN – Biological Rhythms Interview of Assessment jn Neuropsychiatry; CQ – circadian quotient; EPDS – Edinburgh Postnatal Depression Scale; IV – Intradaily Variability; pRA - probability of transitioning from rest to active state; QIC – Quasilikelihood under the Independence Model Criterion; Std Err – Standard Error; TST – Total Sleep Time; μ_A mean activity during active state; μ_R mean activity during rest state.

Supplementary Table 4: Using Generalized Estimating Equations to Model Anxiety Symptoms (GAD7) as Function of the Most Predictive Sleep and Biological Rhythms Variables Across Perinatal Period (n=73)

Variable	ß	Std Err	P Value
Intercept	-9.22	6.03	0.13
Visit 2 (1-3 Weeks Postpartum)#	7.87	7.86	0.32
Visit 3 (6-12 Weeks Postpartum)#	**22.11**	**6.90**	**0.0014**
Age	-0.15	0.096	0.11
Years of Education	0.15	0.15	0.30
BMI	0.078	0.055	0.16
Past History of Mood Disorders (Yes)	0.28	0.97	0.77
Past or Current Anxiety Disorder (Yes)	**1.85**	**0.85**	**0.030**
BRIAN	**0.20**	**0.055**	**0.00028**
Visit 2:BRIAN	0.043	0.065	0.51
Visit 3:BRIAN	-0.034	0.056	0.55
μ_R night	**15.95**	**7.36**	**0.030**
Visit 2: μ_R night	**-18.92**	**7.50**	**0.012**
Visit 3: μ_R night	-17.01	8.88	0.055
pRA Night	14.27	14.98	0.34
Visit 2: pRA Night	**-41.69**	**17.38**	**0.016**
Visit 3: pRA Night	**-56.18**	**18.66**	**0.0026**
MLiT500	0.26	0.34	0.45
Visit 2: MLiT500	-0.32	0.51	0.54
Visit 3: MLiT500	**-1.07**	**0.43**	**0.012**

Model QIC: 561.36; **QICu:** 558.30. #Reference level: Third Trimester of Pregnancy. **Abbreviations:** BMI – Body Mass Index; BRIAN – Biological Rhythms Interview of Assessment jn Neuropsychiatry; GAD7- Generalized Anxiety Disorder- 7; MLiT500 - Mean timing of light exposure above 500 lux; pRA - probability of transitioning from rest to active state; QIC – Quasilikelihood under the Independence Model Criterion; Std Err – Standard Error; TST – Total Sleep Time; μ_R mean activity during rest state.