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Sleep, Reproduction, and Pregnancy

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A Dissertation Presented

by

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ABSTRACT

SLEEP, REPRODUCTION, AND PREGNANCY

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Sleep, and particularly sleep timing, prior to conception may be important for reproductive and pregnancy health. Evidence of a biological mechanism relating sleep to reproductive health from laboratory studies suggests that sleep may influence the hypothalamic-pituitary-ovarian (HPO) axis. The role of sleep in the HPO axis may also impact many reproductive outcomes including reproductive hormones, ovulation, pregnancy, live birth, pregnancy loss, and adverse pregnancy outcomes. However, epidemiological evidence for associations between sleep, especially sleep timing, and these reproductive health endpoints is limited with most studies typically assessing shift work, and not sleep patterns directly. To address these gaps, we evaluated preconception sleep in association with reproductive hormone panels, anovulation, time-to-pregnancy, live birth, pregnancy loss, and adverse pregnancy outcomes. We used data from 1,228 reproductive aged women with 1-2 prior pregnancy losses in the Effects of Aspirin in Gestation and Reproduction (EAGeR) preconception cohort. Preconception sleep duration, timing, and shift work were measured at baseline via self-report. Reproductive outcomes were assessed prospectively over follow-up. We found weak associations between measures of sleep duration, timing, and shift work with reproductive hormones, but only later sleep timing and shift work were associated with greater risk of anovulation. Longer sleep duration, and later sleep timing were associated with longer time-to-pregnancy, but preconception sleep characteristics were not associated with live birth. Preconception sleep was not associated with risk of pregnancy loss, but later sleep timing was associated with risk of adverse pregnancy outcomes. Together, our findings identify sleep during the preconception period as a potentially modifiable risk factor to improve women's reproductive health and time-to-pregnancy, and to reduce adverse pregnancy outcomes. Our findings may provide preliminary evidence to suggest that preconception sleep is not associated with pregnancy loss or live birth.

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CHAPTER 1

SLEEP AND OVULATORY FUNCTION AMONG EUMENORRHEIC, REPRODUCTIVE-AGED WOMEN

Introduction

Current guidelines for sleep recommend that adults achieve 7-9 hours of sleep per night (1, 2). However, good sleep also requires sleeping in line with one's chronotype (the tendency to be a morning or evening person) (1). Not sleeping in line with one's chronotype and having different sleep timing between weekdays and weekends creates a jet lag effect ("social jet lag") and can disrupt biological rhythms leading to diabetes, atherosclerosis, and depression (3-7). Importantly, an estimated 35% of adults do not meet recommended daily sleep duration guidelines, and 69% report having at least one hour of social jet lag (8-11).

Short sleep duration and improper sleep timing induces sleep debt and circadian disruption, which can lead to poor cardiometabolic health and potentially reproductive dysfunction (5, 7, 12-14). Sleep may be related to reproductive function through mechanisms that regulate sleep variability and circadian rhythms. The two-process model of sleep suggests that 1) sleep homeostasis, a compensatory response that regulates variability in sleep and wakefulness, as well as 2) circadian rhythms, which coordinate the timing of physiological processes, regulate the sleep-wake cycle (15-18). Of particular interest are the central and peripheral molecular clocks, which act as circadian pacemakers that keep a consistent timing of physiological processes throughout the body via neuroendocrine signaling, including the timing of sleep-wake rhythms (17, 19-21). Melatonin, a hormone made in the pineal gland, is also responsible for regulating circadian processes in the sleep-wake cycle (22). Sleep duration, and timing, influence these key intermediates of the circadian rhythms pathway (3, 17). Both melatonin and peripheral molecular clocks, are located in the hypothalamus and ovarian tissue and therefore imply a role of sleep in hypothalamic-pituitary-ovarian (HPO) axis regulation (22-30). Indeed, awakening from sleep modulates luteinizing hormone signaling and may imply a role of sleep and wakefulness in regulating reproductive hormone signaling (31). Collectively, these data suggest a pathway by which sleep could impact reproductive hormone signaling and clinically relevant endpoints of HPO axis function including anovulation.

Consistent with laboratory data, a few epidemiologic studies suggest that sleep deprivation and circadian disruption through shift work are associated with reproductive health outcomes including menstrual cycle length, fertility, and miscarriage (32, 33). Further, a few studies reported that acute sleep deprivation, and short sleep duration, are associated with reproductive hormones (34-36). However, these studies only assessed 1-2 reproductive hormones in either the follicular or luteal phase of the menstrual cycle and it is important to evaluate reproductive hormones throughout the menstrual cycle to capture key phases of variability and feedback mechanisms between reproductive hormones. Studies that examine a panel of reproductive hormones can better contextualize how reproductive hormones mutually influence each other and how they are related to ovulatory function. One study that examined sleep and a reproductive hormone panel reported a weak association of sleep duration ≥8 hours and greater follicular phase follicle stimulating hormone, but not luteinizing hormone, estrogen, or progesterone (37). In contrast, the BioCycle study of n=259 premenopausal women, observed no strong association between sleep duration and follicle stimulating hormone, but reported that increasing sleep duration was associated with elevated estrogen and luteal progesterone (38). Importantly, no prior studies have evaluated social jet lag, or mild circadian disruption, with reproductive hormone profiles outside the context of shift work. Previous work has suggested social jet lag is related to premenstrual symptom severity and thus may also influence menstrual cycle function (39).

Though epidemiologic evidence on sleep and anovulation, a clinically meaningful endpoint of HPO axis function, remains understudied. A single study reported that longer sleep duration, and earlier sleep midpoints were potentially associated with greater risk of anovulation, but shift work was not (38). Importantly, this study used shift work as a proxy exposure to characterize extreme circadian disruption among shift workers only. However, it did not capture social jet lag and it remains unclear if mild circadian disruption from social jet lag could influence the reproductive hormonal milieu and ovulation. As such, studies examining social jet lag and risk of anovulation are needed.

Considering the prevalence of sleep duration <7 hours, combined with sparse data on social jet lag, we evaluated whether multiple sleep behaviors (i.e., usual sleep duration, sleep midpoint, social jet lag, and shift work) are associated with subtle perturbations in reproductive hormone signaling throughout the

HPO axis in a large cohort of healthy, eumenorrheic women of reproductive age. In addition, we also evaluated whether these sleep behaviors are subsequently associated with risk of anovulation.

Methods

Participants

This is a secondary analysis of a cohort nested within the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial (40, 41). Inclusion and exclusion criteria and full details for the EAGeR study are previously described. Briefly, EAGeR (n=1228) was a block-randomized, double-blind, placebo controlled trial conducted in the US including women recruited from the states of Utah, New York, Pennsylvania, and Colorado from 2006-2012. Women must have had 1-2 prior pregnancy losses within the past 12 months, have intact uterus, tubes, and ovaries, be aged 18-40 years at baseline, and have regular menstrual cycles (21-42 days length). Women must not have been pregnant at baseline, and were actively trying to conceive. Women were excluded if they had contraindications for non-steroidal anti-inflammatory drugs (NSAIDs), presence of major medical disorders, history of infertility, or were undergoing assistive reproductive technologies.

Women were followed for up to 6 cycles while trying to become pregnant and were followed throughout pregnancy if they conceived. Fertility monitors (ClearBlue Easy; Inverness Medical, Waltham, MA) were distributed to participants in order to provide information on timing of ovulation to improve likelihood of conception and to aid in clinic visit scheduling (41). These fertility monitors measure urinary luteinizing hormone and estrone-3-glucuronide. Pregnancy was determined by positive urinary β-hCG (human chorionic gonadotropin) pregnancy tests (Quidel, San Diego, CA). The study was approved by the Institutional Review Board at each study site and data coordinating center. All participants gave informed consent prior to study entry.

Among n=1228 women in EAGeR, 14 were missing data from fertility monitors to assess ovulation and 14 withdrew and thus did not contribute cycles of follow-up. This left an analytic sample of 1200 women with 3784 cycles at risk of anovulation contributed over follow-up. Of the 1200 women, 22.3% (267/1200) became pregnant in the 1st cycle of follow-up, and 1.25% (15/1200) were lost to follow-up at the end of the 1st cycle. Of these women, four did not have reproductive hormone data available. This left n=914 participants that were included in our analytic sample for reproductive hormone analyses.

Assessment of Sleep Characteristics

Sleep characteristics including usual, average bedtime, wake time, and sleep onset latency were measured at baseline via self-report for both weekdays and weekends. Using these self-reported characteristics we calculated: 1) sleep duration, 2) sleep-debt corrected midpoint of sleep on "free" days, and 3) social jet lag. Sleep duration was calculated as the difference in usual time to get up and bedtime and subtracting the amount of usual time to fall asleep for weekdays and weekends. Sleep duration was weighted for weekday (5/7) and weekend (2/7) contributions. We evaluated two sleep timing measures: sleep midpoint and social jet lag. We calculated sleep midpoint as the midpoint of an average weekend sleep interval (the midpoint between sleep onset and waking) and subtracting half the difference between weekend and total sleep durations in order to correct for sleep debt accrued over weekdays (3, 42, 43). Specifically, sleep debt is assumed to be accrued over weekdays and may be greater for individuals with greater tendency to be evening persons (later sleep midpoints) because typical workdays would impact their need for sleep on the weekends. This sleep debt is compensated for greater "free day" sleep on weekends assuming they are free from the influence of an alarm clock. Thus, correcting sleep midpoints for sleep debt provides a better proxy of chronotype, the biological construct of "temporal phenotype" (morning type or evening type), than weighting sleep midpoint for weekdays and weekends (3, 42, 43). We applied the sleep debt correction to sleep midpoint given the cohort included both employed and unemployed women and the inability to distinguish when individuals are free from work or other responsibilities that may impact sleep midpoint. For individuals who sleep longer on weekdays than weekends, we did not correct their weekend sleep midpoints for sleep debt (3). We also evaluated social jet lag, or the discrepancy in usual sleep timing between work days and "free" days (4). This was accomplished by taking the absolute difference between weekend and weekday sleep midpoints and comparing this in categories <1, 1-2, and ≥ 2 hours as has been done in prior literature (3, 4, 6). In calculating social jet lag, we assume that weekends were "free" days, or days in which individuals were free from work and the influence of an alarm clock, as we did not have detailed information on work schedules (4, 6). Shift work was assessed at baseline by asking if participants had night shift work and rotating shift work schedules for their most recent job (current or past employment). Additional details on sleep variable assessment and operationalization are shown in Table 1.1.

Assessment of Reproductive Hormones and Anovulation

Urinary reproductive hormones, including follicle stimulating hormone (FSH), luteinizing hormone (LH), estrone-1-glucuronide (E1G), estradiol (E2), and pregnanediol-3-glucuronide (PDG) were assayed 3-8 times per cycle during the first two menstrual cycles. Most participants (90%) were assayed 3-4 times per cycle in order to collect reproductive hormones from specific phases of the menstrual cycle (one sample in the follicular phase, one on the expected day of ovulation, and two during the luteal phase). Additional samples (up to 8) were measured in cycles where no LH peaks were observed or in long cycles to capture unique hormonal fluctuations (10%). Urinary FSH and LH were assessed via reagent/sandwich immunoassay (Roche Diagnostics, Indianapolis, IN), and E2, E1G and PDG were measured by competitive chemiluminescence duplex assay (Quansys Biosciences, Logan, UT). The interassay laboratory coefficients of variation for FSH, LH, E2, E1G, and PDG were 1.8%, 1.6%, 13.7%, 20%, and 23% respectively.

Anovulation is the probability that a given cycle is not ovulatory as defined by low luteal progesterone and mid-cycle LH values (44). Participants contributed data for up to 6 menstrual cycles, or until hCG-detected pregnancy, or drop out, whichever came first. We classified the anovulatory status of menstrual cycles using the following criteria, which were adapted from Behre et al. and has 90.6% agreement with ultrasound detected ovulation (the gold standard) (45). All cycles resulting in hCG-detected pregnancy were classified as ovulatory. Cycles in which no pregnancy occurred, but had maximum PDG $\geq$5 μg/mL (1st two cycles only) or with LH values >2.5 times the average of the prior 5 days as measured via fertility monitors, or with a "peak" fertility monitor reading, which indicates an LH surge, were classified as ovulatory (45-47). Urinary progesterone values were used to improve ovulation detection sensitivity in cycles 1-2. Cycles not meeting these criteria were defined as anovulatory.

Assessment of Covariates

Participants completed baseline questionnaires assessing demographics, lifestyle, and substance use history. Weight (kg) and height (m) were measured using standardized protocols and were used to calculate body mass index (BMI). Smoking status was measured via baseline questionnaire and urine cotinine biomarkers. Sleep aids and melatonin supplement use over the past 12 months were assessed at baseline via self-report. Marijuana, opioid, and antidepressant metabolites were measured from urine samples collected at baseline. Urine samples were assayed using the Drug of Abuse IV Ultra

chemiluminescent immunoassay measured on the Evidence Investigator (Randox Toxicology, County Antrim, United Kingdom). Positive screens were indicated based on standard manufacture-based cutoffs.

Statistical Analysis

We summarized participant characteristics and sociodemographics by sleep duration and sleep midpoint among women in our analytic sample (n=1200). Hourly categories for sleep duration (hours; <6, 6-7, 7-8 (referent), 8-9, and ≥9) were chosen given we were interested in investigating associations at shorter (<6 hours) and longer (≥9 hours) sleep durations. Sleep midpoint (tertiles; tertile 1, tertile 2 (referent), tertile 3) was analyzed as tertiles given the lack of population-appropriate cut points for early, normal, and late sleep midpoints for reproductive aged women. Social jet lag (hours; 0-1 (referent), 1-2, ≥2) was analyzed as hourly levels. Rotating shift work (vs. non-rotating shift work) and night shift work (vs. non-night shift work) were assessed as binary variables. Missing exposure and covariate information was addressed using multiple imputation in all analyses across n=10 datasets using 20 burn-in iterations (48).

We estimated crude risk ratios (RR) and 95% confidence intervals (CI) for associations between sleep behaviors and risk of anovulation using generalized estimating equations (GEE) with log-Poisson distributions and accounted for repeated menstrual cycles using unstructured correlation matrices. We used unstructured covariance matrices given we wanted to explicitly estimate the covariance between each cycle as the anovulatory status of one cycle may be sporadic or dependent on the prior cycle (49, 50). We also estimated crude geometric mean differences and 95% CIs between sleep characteristics and log-transformed reproductive hormones using GEEs of linear models. We transformed linear coefficients of reproductive hormone geometric mean differences to a percentage difference (PD) scale using ((exp(β coefficient)-1)*100). We used a first-order autoregressive (AR(1)) covariance matrix structure to handle repeated measures from each participant nested within two cycles of follow-up. This covariance structure was used given we assume that the correlation between reproductive hormones will become weaker over successive measures (49). We examined FSH, E2, and E1G across all menstrual cycle phases. For LH and PDG, these reproductive hormones have specific biological functions relevant to a given phase. Thus, LH was examined only on the expected day of ovulation and PDG was examined only in the luteal phase. We weighted all models of anovulation and reproductive hormones using the inverse of menstrual cycles each

participant contributed (1/#menstrual cycles contributed). These weights were used to account for dropout and women who became pregnant during follow-up, which would affect their number of contributed cycles at risk of anovulation (51). We additionally controlled for confounding in multivariate models using *a priori* specified confounders including age, BMI, parity, stress, opioid use, antidepressant use, marijuana use, race, education, employment, smoking, exercise, alcohol use, caffeine intake, season, and sleep aid use. In addition, models of reproductive hormones included polynomial terms of standardized cycle day (t, t^2, t^3, t^4), which were used to mimic hormone curvature over a menstrual cycle (52). EAGeR was originally conducted as a randomized control trial with half of participants randomized to receive low dose aspirin (81 mg) and folic acid (400 mcg) and the other half of participants randomized to receive a placebo and folic acid (400 mcg) (40, 41). Treatment arm assignment was not associated with sleep characteristics, and thus was not considered a confounder in our associations.

Our determination of anovulatory cycles was based on a classification algorithm developed by Behre et al (45). This classification approach was modified to incorporate urinary luteal progesterone values in the first two cycles of follow-up when luteal progesterone measures were available. We anticipated our method for determining anovulatory cycles as described above would perform better at classifying the outcome among the first two cycles when progesterone values were incorporated. As such, we conducted a sensitivity analysis restricting our analyses of anovulation to the first 2 cycles (n=2010 cycles) to test this hypothesis. All statistical analyses were conducted using SAS v 9.4 software (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics

Among women in EAGeR with data on ovulation and sleep characteristics, (n=1192) most slept 7-8 hours (39.0%) or 8-9 hours (35.2%), and fewer women slept ≥9 hours (11.0%), 6-7 hours (11.0%), or <6 hours (3.78%). Compared to women with 7-8 hours sleep, women who slept ≥9 hours were younger and had lower BMI. These women were more likely to be unemployed and were more likely to use opioids, marijuana, antidepressants, and sleep aids compared to women with 7-8 hours sleep (Supplementary Table 1.1S). Compared to those who slept 7-8 hours, both those with sleep <6 hours and ≥9 hours were more likely to work as night and rotating shift workers and were more likely to smoke. Compared to women who

slept 7-8 hours, women with <6 hours sleep duration were more likely to have children. The majority of women tended to have sleep midpoints <5:00AM with the medians of sleep midpoint tertiles being 2:45AM for tertile 1 (interquartile range (IQR): 2:22AM-3:00AM), 3:36AM for tertile 2 (IQR: 3:25AM-3:48AM), and 4:40AM for tertile 3 (IQR: 4:15AM-5:16AM). Women with sleep midpoints in the 1st and 2nd tertile tended to be older, have greater parity, lower social jet lag, and were less likely to use opioids and marijuana compared to the 3rd tertile. Average sleep duration was longer among sleep midpoints in the 3rd tertile (Supplementary Table 1.2S). Among women in EAGeR, 2.9% (34/1192) of individuals slept later on weekdays than weekends, 71.4% (851/1192) slept later on weekends than weekdays and 25.8% (307/1192) had the same timing of sleep on weekdays and weekends.

Anovulation

Overall, 13% (496/3784) of cycles were classified as anovulatory (Table 1.2). Sleep duration was not associated with risk of anovulation (Table 1.2). Rotating shift work (vs. non-rotating shift work; RR: 1.15, 95% CI: 0.90, 1.47) and night shift work (vs. non-night shift work; RR: 1.20, 95% CI: 0.96, 1.50) were associated with higher risk of anovulation, though neither were statistically significant. Compared to the 2nd tertile of sleep midpoints, later sleep midpoints were associated with greater risk of anovulation (3rd RR: 1.29, 95% CI 1.02, 1.63), whereas earlier sleep midpoints were not (1st tertile RR: 0.90, 95% CI 0.70, 1.15). Social jet lag was not associated with risk of anovulation (Table 1.2).

In sensitivity analyses restricted to the first two cycles, associations between night shift and rotating shift work and risk of anovulation were stronger in magnitude (night shift work vs. not night shift work; RR: 1.38, 95% CI: 1.07, 1.76; rotating shift work vs. not rotating shift work; RR: 1.40, 95% CI: 1.07, 1.84) than when including all cycles. Results for sleep midpoint and risk of anovulation were similar in direction, but weaker in magnitude of association (3rd vs. 2nd tertile; RR: 1.19, 95% CI 0.92, 1.55; Table 1.2).

Reproductive Hormones

Sleep characteristics including sleep duration, shift work, and sleep timing, were not associated with many reproductive hormone levels, but were associated with some minor perturbations in specific reproductive hormones. Sleep duration <6 hours was associated with elevated FSH (vs. 7-8 hours; PD:

35%, 95% CI: 10%, 66%; Table 1.3) and elevated ovulatory LH (vs. 7-8 hours; PD: 63%, 95% CI: 2%, 161%), though sleep duration was not associated with other hormones.

When considering shift work, rotating shift work was associated with lower ovulatory LH (vs. non-rotating shift work PD: -21%, 95% CI: -37%, 0%) and lower luteal PDG (vs. non-rotating shift work PD: -14%, 95% CI: -26%, 1%; Table 1.4). Night shift work (vs. non-night shift work) was also associated with 19% lower luteal PDG (95% CI: -29%, -7%) as well as 13% lower E1G (95% CI: -21%, -4%), but not other reproductive hormones. Sleep timing measures were also not strongly associated with many reproductive hormones. Only the earliest sleep midpoints were associated with a weakly elevated E2 (1^{st} tertile vs. 2^{nd} tertile; PD: 10%, 95% CI: 3%, 17%; 3^{rd} tertile vs. 2^{nd} tertile; PD: -1%, 95% CI: -8%, 5%), but not any other reproductive hormones (Table 1.5). Later sleep midpoints were not associated with any reproductive hormones (Table 1.5). Greater social jet lag was marginally associated with lower FSH (≥2 vs 0-1 hours PD: -11%, 95% CI: -23%, 1%; Table 1.6).

Discussion

In this cohort of eumenorrheic women of reproductive-age, rotating and night shift work were associated with greater risk of anovulation, and with decreases in ovulatory LH, luteal PDG, and E1G. There was also some suggestion that sleep timing may be important as early sleep midpoints were associated with an increase in E2 and later sleep midpoints were associated with greater anovulation risk. In contrast, sleep duration, and social jet lag were not associated with risk of anovulation, but were weakly associated with some reproductive hormone perturbations.

Our null results for sleep duration and anovulation are generally consistent with those reported in BioCycle, a study of 259 reproductive-aged women. However, in BioCycle acute sleep duration <7 hours was potentially associated with an increased risk of anovulation (vs. ≥7 hours; RR: 1.63, 95% CI: 0.84, 3.16), though the results may be imprecise due to limited power (38). The BioCycle study used repeated measures of sleep duration leading up to the expected LH surge in each cycle. Therefore, the measures in BioCycle may capture acute rather than habitual effects of sleep duration on anovulation and may answer questions as to whether sleep on the night before is associated with expected ovulation. In contrast, we answer a different question in EAGeR by examining habitual sleep duration and whether on average usual, sleep duration may be related to a potentially anovulatory cycle. When considering our results for

reproductive hormones, we noted associations between sleep duration <6 hours and greater FSH and LH. It is plausible the magnitude of these changes may not have been sufficient to lead to anovulation. Compared to studies evaluating hormone concentrations, our findings agree with results reported from a cohort of 106 eumenorrheic women, in which sleep duration ≥8 hours/day was correlated with greater follicular FSH levels (37). However, our specific associations between sleep duration and FSH differ in direction, but generally support a role of sleep in reproductive hormone perturbations. The minor differences in our results may be due to population differences and that this study of 106 eumenorrheic women had more women (60.4%) sleeping longer than 8 hours compared to EAGeR (46.2%) (37). Our findings for sleep duration and reproductive hormones are weakly consistent with those reported in BioCycle. Both studies suggest that sleep duration is associated with mild perturbations in reproductive hormone concentrations, though each study identified associations with different hormones. Importantly, these studies asked different questions, with EAGeR focused on habitual sleep and BioCycle focusing on sleep the night before hormone measurement. In BioCycle, sleep duration was not associated with FSH, though the greater sleep duration was associated with elevated E2 and luteal PDG levels (38). The differences between our findings may be due to measurement of acute rather than habitual sleep duration in BioCycle (38). Together, this evidence suggests that usual sleep duration may be associated with some perturbations in reproductive hormones, but not with anovulation risk. Though, with some inconsistencies among findings in these studies and few strong associations, further study using objective, repeated measures of sleep duration could help to better characterize both usual and acute associations of sleep duration with reproductive hormones and anovulation.

We also report on associations between night and rotating shift work and greater risk of anovulation. Our findings are consistent with reports of shift work and greater menstrual cycle irregularity (33), but contrast associations reported in BioCycle, in which night shift work was not associated with anovulation risk (vs. non-night shift work; RR: 0.70, 95% CI: 0.32, 1.56) (38). However, BioCycle was limited by the few shift workers (n=77) included in the analyses and may have been underpowered to detect associations. Rotating and night shift work were both associated with lower ovulatory LH, luteal PDG, and E1G. Although, rotating shift work was marginally associated with luteal PDG and may have been an issue with power. The findings of associations between shift work and lower reproductive hormones (LH, PDG,

and E1G) supports our findings for shift work and anovulation risk. Our findings are generally in agreement with associations of shift work and reproductive hormones reported in other studies. Shift work was associated with hormonal perturbations of different hormones in each study and it could be these differences may be due to how hormones were measured at different times in the menstrual cycle compared to those in EAGeR. Of these studies, which include premenopausal shift workers in occupational cohorts, shift work has been associated with greater luteal FSH and LH (53), and greater estradiol, but not progesterone (54). Longer duration of night shift work was associated with elevated estradiol (53, 55). An important limitation of these studies was that they investigated select reproductive hormones rather than a full reproductive hormone panel and it is unclear what associations may have been present for unmeasured reproductive hormones in each study. To our knowledge, BioCycle is the only other study that comprehensively evaluated night shift work with reproductive hormones and observed no associations with FSH, LH, PDG, or E2 (38). The discrepancies between our results and those reported in these studies may be due to recency and duration of shift work (38, 53-55), as well as collection of urine samples during the luteal phase only (55). Taken together, the literature on shift work and reproductive hormones suggests shift work is associated with reproductive hormone perturbations, but associations with specific hormones remain unclear.

In considering sleep midpoint and risk of anovulation, our results potentially suggest later sleep midpoints may be associated with greater risk of anovulation. Though, our results generally contrast those reported in the BioCycle study, in which early sleep midpoints were associated with greater risk of anovulation (RR: 2.50, 95% CI: 0.93, 6.77), but later sleep midpoints were not (RR: 1.20, 95% CI: 0.50, 2.86) (38). However, these associations may have been limited in power due to the small number of anovulatory cycles accrued in the BioCycle study (38). When considering reproductive hormones in EAGeR, we observed that later sleep midpoints were not significantly associated with any of the reproductive hormones in the hormone panel. Though, among early sleep midpoints, we observed a weak increase in estradiol (1^{st} vs. 2^{nd} tertile). Our finding for early sleep midpoints and estradiol is consistent with a study of premenopausal nurses, which reported that earlier sleep midpoints were weakly correlated with higher estradiol levels (56). Though, the BioCycle study did not observe any associations between sleep midpoints and a longitudinally assessed reproductive hormone panel (38). Many women in EAGeR

tended to have early sleep midpoints and it is plausible we may have sufficient power to detect associations between earlier sleep midpoints and estradiol compared to BioCycle which had more women with later sleep midpoints (38, 57-59). Conversely, this may have hindered our detection of associations between later sleep midpoints and reproductive hormones. With the current state of the literature, there may be a weak association between later sleep midpoints and risk of anovulation, though additional work is needed using actigraphy-measured sleep midpoints to further explore the role of acute mild fluctuations in sleep midpoints on these outcomes.

Our study adds to the sleep timing literature by characterizing the association of social jet lag with reproductive hormones and risk of anovulation. Overall, social jet lag was not associated with a greater risk of anovulation, but was weakly associated with marginally lower FSH. This may suggest that mild circadian disruption from differences in usual weekday-weekend sleep timing may not strongly influence HPO axis function compared to strong circadian disruption from shift work. To our knowledge, only one prior study characterized social jet lag and progesterone among reproductive aged women, but was limited to progesterone assessed via hair samples and did not consider a comprehensive reproductive hormone panel (60). Therefore, our work is not completely comparable to the evidence presented within this study. With few studies considering social jet lag, further study is needed to replicate our findings using repeated, objective sleep assessments of social jet lag in association with reproductive hormones and risk of anovulation.

Sleep is hypothesized to be related to HPO axis function through complex pathways in which mechanisms are not fully understood. Sleep behaviors influence the physiological processes of sleep, composed of 1) sleep homeostasis and 2) circadian rhythms that constantly interact to control the sleep-wake cycle and may influence reproductive hormone signaling (15-17, 22-30). The physiological sleep state may directly influence reproductive hormone signaling through modulation of the ultradian rhythm of gonadotropin releasing hormone (GnRH) pulsatility independent of time of day (31). Slowing of the GnRH pulse through sleep onset affects FSH and LH signaling with most studies noting lower concentration and longer pulse of LH, though mixed evidence of higher or lower FSH, which may be dependent upon LH concentration (31, 61, 62). This may explain why shorter sleep duration was associated with elevations in both FSH and LH.

The circadian rhythms component of the two-process model of sleep involves molecular clocks that control the timing of many processes throughout the body (21). Genetic variation in molecular clocks at the population level has resulted in individuals who respond differently to the same light-dark cycle and thus make up a range of chronotypes (3). Evidence from animal models suggests that a central circadian molecular clock in the hypothalamus regulates both sleep-wake physiology and GnRH release in the HPO axis (63). This central molecular clock is also linked to peripheral clocks throughout the body via timed feedback loops involving core clock genes such as Bmal1:Clock, and Per:Cry that influence timing of processes in other tissues (63, 64). For example, molecular clocks are found in both theca and granulosa cells in the ovary and it is hypothesized these mechanisms regulate sensitivity to the LH surge and prostaglandin synthesis to initiate ovulation (64). Given chronotypes vary in clock genes and later chronotypes have been associated with longer menstrual cycles and shifts in the timing of hormone peaks, variation in clock genes or their expression may explain why some chronotypes may have delayed or disrupted ovulatory function (38, 65-67). Night shift work may result in misalignment of biological rhythms and altered clock gene expression and signaling, which could disrupt timing of key menstrual cycle processes including estrogen synthesis (68, 69). Therefore, clock genes and their expression may be intermediates of the associations between sleep midpoint and shift work and greater risk of anovulation.

Strengths and Limitations

Our study has many strengths including longitudinal reproductive hormones assessments timed to relevant cycle phases and identification of anovulatory cycles using a highly sensitive algorithm. In addition, we used weighting to address differential contribution of menstrual cycles due to women dropping out or becoming pregnant over follow-up. Our study also has a few limitations. We did not use ultrasound-based assessments to capture anovulatory cycles given ultrasonography is impractical to use in a large prospective cohort. Rather, we used a robust, high-sensitivity algorithm incorporating luteal progesterone measurements during the first two cycles that has 90.6% agreement with ultrasound based measurements (45). When we restricted to the first two cycles to determine whether associations differed due to incorporation of luteal progesterone measurements, the associations between shift work and anovulation were stronger in magnitude while associations between later sleep midpoints and anovulation were somewhat attenuated. This may suggest that estimation of the point estimates is somewhat sensitive to

differences in the anovulation classification method though it should be noted that overall similar conclusions were reached. Further research into the mechanism of night shift work and ovulatory function is warranted.

Several studies suggest a role of sleep in reproductive hormone perturbations over the menstrual cycle and vice versa, that reproductive hormones may acutely influence sleep over menstrual cycle phases (31, 34, 35, 70-81). Indeed, acute changes in sleep may have time-varying associations with reproductive hormones that we were not able to capture in this study as sleep characteristics were based on a single baseline measurement. As such, our inference is limited to habitual or usual sleep characteristics. However, our baseline measure of sleep, followed by measurement of reproductive hormones in cycles 1 & 2, and anovulation for up to 6 cycles, ensures temporality of these associations. It is possible that baseline assessment of usual sleep characteristics may not represent the most appropriate etiologically relevant time period for anovulation in later cycles of follow-up, though evidence suggests that most individuals (76.5%) report consistent trajectories of usual sleep duration over a much longer period of time for up to four years (82). Thus, it is likely that most participants sleep schedules remained quite similar over the six month follow-up in our study. It is currently unknown whether usual sleep characteristics change among couples attempting to conceive. Our measures of sleep midpoint and social jet lag were based on calculations using the sleep midpoints of weekend and weekday sleep and assuming that weekend sleep midpoints represent the midpoints of days "free" from work or the influence of an alarm clock (4, 6). Unfortunately, we did not have information on work or school schedules to determine whether weekends were in fact "free days." Of note, our measurement of rotating and night shift work was based on participant self-report of most recent job and may not reflect their current work schedule, which may introduce some measurement error.

Our study evaluated multiple sleep domains with a panel of reproductive hormones and results should be interpreted cautiously given the potential for type one error due to multiple comparisons. However, we reported on reproductive hormones associations to further contextualize our associations of sleep and anovulation. The EAGeR population was comprised of mostly healthy, regularly cycling, white, pregnancy planning women with a history of pregnancy loss, which may limit the generalizability of our results. Though, almost 30% of reproductive age women have experienced a prior pregnancy loss and

represent a large proportion of women to whom these results would be generalizable (83). Future research among diverse populations is warranted.

Conclusions

Our results suggest that sleep duration is not strongly associated with reproductive hormone fluctuations or risk of anovulation. Shift work and potentially later sleep midpoints may be associated with greater risk of anovulation and that shift work may be weakly associated with disrupted HPO axis function. Our work importantly characterized social jet lag and suggested that mild circadian disruption from social jet lag was associated with some perturbation in FSH, but was not associated with risk of anovulation. However, there are still remaining questions as to whether objective, and repeated measures of sleep midpoint and social jet lag may elucidate acute, day-to-day changes in the reproductive hormonal milieu and HPO axis function. Thus, further studies in premenopausal women are needed using actigraphy or gold standard measures of sleep to evaluate chronotype and social jet lag prospectively in association with reproductive hormones and risk of anovulation.

CHAPTER 2

SLEEP DURATION, SLEEP TIMING, SHIFT WORK, AND FECUNDABILITY

Introduction

Sleep is a critical process that is important for cognition, immune system modulation, and potentially fertility (24, 84). Short sleep duration and improper sleep timing has consequences on sleep-wake physiology by influencing sleep homeostasis and by altering endogenous circadian clock mechanisms, which may be linked with fertility through the hypothalamic pituitary ovarian (HPO) axis and menstrual cycle function (17, 21, 69, 73, 85-91). While there is epidemiological evidence to support that disruptions to sleep are associated with perturbations in the reproductive hormonal milieu, disrupted menstrual cycle length, and sporadic anovulation, how sleep is related to downstream reproductive endpoints, such as fecundability remains unclear (24, 33, 34, 37, 38, 53, 92, 93).

Epidemiological evidence on sleep and fecundability is scarce. To date, evidence of an association between sleep and fecundability has come from the Pregnancy Study Online (PRESTO), a large, preconception cohort that reported sleep duration <6 hours and poor sleep quality were associated with reduced fecundability (94). Importantly, less is known regarding sleep timing measures and fecundability. Instead, shift work has been used to characterize circadian disruption and its association with fecundability (94-100). This literature has been inconsistent, with evidence of delayed fecundability among shift workers where fecundability was retrospectively recalled (95-100), and null associations from studies where fecundability was evaluated prospectively (94). However, shift work does not capture direct measures of sleep timing, nor circadian disruption among non-shift workers. Sleep timing measures such as sleep midpoint (a proxy of "chronotype," or the tendency to be a morning person ("morning lark") or evening person ("night owl")), and social jet lag (the difference in sleep timing between workdays and "free" days) may offer more direct measures of sleep timing and its role with fecundability (3, 4, 57). However, neither measure has been robustly evaluated with fecundability, though both have been related to pregnancy difficulties and severe menstrual symptoms, respectively (39, 67). Thus, sleep midpoint and social jet lag may offer important insights into the role of sleep timing with fecundability.

Therefore, our objective was to evaluate the role of sleep characteristics on fecundability through multiple sleep measures including sleep duration, sleep midpoint, social jet lag, and shift work. Many

reproductive aged women in the US do not meet recommended sleep guidelines, and this work is needed to understand the role of potentially modifiable influences, such as sleep, on fecundability (9, 101).

Methods

Participants

This is a secondary analysis of a cohort nested within the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial (40, 41). Inclusion and exclusion criteria and full details are previously described. Briefly, EAGeR (n=1228) was a block-randomized, double-blind, placebo controlled trial conducted in the US including women recruited from the states of Utah, New York, Pennsylvania, and Colorado from 2006-2012. Women had a history of 1-2 prior losses, and must have had an intact uterus, tubes, and ovaries, and be aged 18-40 at baseline. Women must have had regular menstrual cycles (21-42 days length), must have not been pregnant at baseline, and were actively trying to conceive. Women were excluded if they had contraindications for NSAIDs, presence of major medical disorders, history of infertility, or were undergoing assistive reproductive technologies. Women were followed for up to 6 cycles while trying to become pregnant and throughout pregnancy for those who became pregnant (n=797). Fertility monitors (ClearBlue Easy; Inverness Medical) were used during the preconception cycles to improve timing of intercourse, optimize conception and aid in clinic visit scheduling (41).

Assessment of Sleep Characteristics

Sleep characteristics including usual, average bedtime, wake time, and sleep onset latency were measured at baseline via self-report for both weekdays and weekends. Using these self-reported characteristics we calculated: 1) sleep duration, 2) sleep-debt corrected midpoint of sleep on “free” days, and 3) social jet lag. Sleep duration was calculated as the difference in usual time to get up and bedtime and subtracting the amount of usual time to fall asleep for weekdays and weekends. Sleep duration was weighted for weekday (5/7) and weekend (2/7) contributions. We evaluated two sleep timing measures: sleep midpoint and social jet lag. We calculated sleep midpoint as the midpoint of an average weekend sleep interval (the midpoint between sleep onset and waking) and subtracting half the difference between weekend and total sleep durations in order to correct for sleep debt accrued over weekdays (3, 42, 43). Specifically, sleep debt is assumed to be accrued over weekdays and may be greater for individuals with greater tendency to be evening persons (later sleep midpoints) because typical workdays would impact their

need for sleep on the weekends. This sleep debt is compensated for greater "free day" sleep on weekends assuming they are free from the influence of an alarm clock. Thus, correcting sleep midpoints for sleep debt provides a better proxy of chronotype, the biological construct of "temporal phenotype" (morning type or evening type), than weighting sleep midpoint for weekdays and weekends (3, 42, 43). We applied the sleep debt correction to sleep midpoint given the cohort included both employed and unemployed women and the inability to distinguish when individuals are free from work or other responsibilities that may impact sleep midpoint. For individuals who sleep longer on weekdays than weekends, we did not correct their weekend sleep midpoints for sleep debt (3). We also evaluated social jet lag, or the discrepancy in usual sleep timing between work days and "free" days (4). This was accomplished by taking the absolute difference between weekend and weekday sleep midpoints and comparing this in categories <1, 1-2, and ≥2 hours as has been done in prior literature (3, 4, 6). In calculating social jet lag, we assume that weekends were "free" days, or days in which individuals were free from work and the influence of an alarm clock, as we did not have detailed information on work schedules (4, 6). Shift work was assessed at baseline by asking if participants had night shift work and rotating shift work schedules for their most recent job (current or past employment). Additional details on sleep variable assessment and operationalization are shown in Table 2.1.

Assessment of Fecundability

We assessed fecundability as time to pregnancy (TTP), which was measured as the discrete number of menstrual cycles required to achieve hCG pregnancy for up to 6 menstrual cycles of consecutive follow-up (102). Pregnancies were ascertained via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses. Pregnancy tests were also conducted from batched augmented testing using daily first-morning urine collected over the first two cycles of follow-up and on spot urine samples from monthly clinic visits. Free β-hCG was measured in these stored urine samples to detect very early, unrecognized pregnancies. Samples were analyzed via sequential laboratory assays for free β-hCG (initial test: Catalog #: RIS0011R, BioVendor, Asheville, NC; confirmatory test: catalog #4221-16, Diagnostic Automation Inc., Calabasas, CA). Pregnancies lasting 6-7 weeks gestation were confirmed via ultrasound.

Assessment of Covariates

Participants completed baseline questionnaires assessing demographics, lifestyle, substance use, and reproductive and familial medical history. Weight (kg) and height (m) were measured using standardized protocols and were used to calculate BMI. Smoking status was measured via baseline questionnaire and urine cotinine biomarkers. Sleep aid and melatonin supplement use over the past 12 months were assessed at baseline via self-report. Marijuana, opioid, and antidepressant metabolites were measured from urine samples collected at baseline. Urine samples were assayed using the Drug of Abuse IV Ultra chemiluminescent immunoassay measured on the Evidence Investigator (Randox Toxicology, County Antrim, United Kingdom). Positive screens were based on standard manufacture-based cutoffs.

Statistical Analysis

At baseline, most women in EAGeR (99.3%, n=1220/1228) reported sleep data with only eight women missing data for usual bedtime or waking time (<1.0%, n=8/1228). As such, we summarized participant characteristics and sociodemographics by categories of preconception sleep duration and preconception sleep midpoint among women with complete sleep data (n=1220). We used discrete Cox proportional hazards models to estimate fecundability odds ratios (FORs) and 95% confidence intervals (CIs), accounting for left truncation as some pregnancy attempts occurred prior to study entry, and right censoring at end of follow-up time. We compared associations in unadjusted and multivariable adjusted models using confounders identified *a priori* via directed acyclic graphs. These potential confounders included age, BMI, parity, stress, opioid use, marijuana use, antidepressant use, race, education, smoking, employment, alcohol, caffeine, season, sexual intercourse frequency in the past month, sleep aid use, and exercise. EAGeR was originally conducted as a randomized control trial with half of participants randomized to receive low dose aspirin (81 mg) and folic acid (400 mcg) and the other half of participants randomized to receive a placebo and folic acid (400 mcg) (40, 41). Treatment arm assignment was not associated with sleep characteristics, and thus was not considered a confounder in our associations.

We examined sleep characteristics as categorical and continuous variables. Hourly categories for sleep duration (hours; <6, 6-7, 7-8 (referent), 8-9, and ≥9) were chosen given we were interested in investigating associations at shorter (<6 hours) and longer (≥9 hours) sleep durations. Sleep midpoint (tertiles; tertile 1, tertile 2 (referent), tertile 3) was analyzed as tertiles given the lack of population-appropriate cut points for early, normal, and late sleep midpoints for reproductive aged women. Social jet

lag (hours; 0-1 (referent), 1-2, ≥2) was analyzed as hourly levels. Linear tests for trend were conducted evaluating continuous sleep duration, sleep midpoint, and social jet lag in association with cumulative pregnancy noting linear associations for sleep midpoint (p=0.02) and social jet lag (p=0.03), but not sleep duration (p=0.45). As such, continuous models are only presented for sleep midpoint and social jet lag. Rotating shift work (vs. non-rotating shift work) and night shift work (vs. non-night shift work) were assessed as binary variables. Missing exposure and covariate information was addressed using multiple imputation in all analyses across n=10 datasets using 20 burn-in iterations (48).

We also performed two sensitivity analyses. First, to account for the potential influence of conception delays, or non-conception over long-term pregnancy attempts, on sleep patterns before entering the cohort, we restricted our analyses to women with ≤3 cycles of prior attempts (n=917) at baseline. We chose ≤3 cycles of prior attempts based on work in other studies of sleep and fecundability (94, 103). Second, women with shift work schedules may have greater disrupted sleep than women in permanent day shifts and thus we restricted our analyses to women who did not report any rotating or night shift work (n=841) to account for any potential residual confounding due to shift work (104). Statistical analyses were conducted using SAS v 9.4 software (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics

At baseline, most women in EAGeR (n=1220) who reported sleep data slept 7-8 hours (38.8%) with slightly fewer women sleeping 8-9 hours (35.2%). Women who slept longer (≥9 vs. 7-8 hours) were younger, had lower BMI, and lower education, but were more likely to use marijuana, antidepressants, and sleep aids (Table 2.2). Women who slept <6 hours (vs. 7-8 hours) were also more likely to have children. Compared to those with 7-8 hours sleep duration, both those with sleep <6 hours and ≥9 hours were more likely to work as night and rotating shift workers, use opioids, and smoke. The majority of women tended to have sleep midpoints <5:00AM with the medians of sleep midpoint tertiles being: 2:45AM (IQR: 2:20AM-3:00AM) for tertile 1, 3:36AM (IQR: 3:25AM-3:48AM) for tertile 2, and 4:40AM (IQR: 4:21AM-5:17AM) for tertile 3. Women with sleep midpoints in the 1st and 2nd tertiles (vs. 3rd tertile) tended to be older, have lower social jet lag, were less likely to use opioids and marijuana, and had greater education, and parity. Average sleep duration was longest among sleep midpoints in the 3rd tertile

(Supplementary Table 2.1S). Among women in EAGeR, 3% (36/1220) of individuals slept later on weekdays than weekends, 70.9% (865/1220) slept later on weekends than weekdays and 26.1% (319/1220) had the same sleep timing on weekdays and weekends.

Fecundability

The median cycles of follow-up until 1st hCG detected pregnancy was 2 (25th, 75th percentiles: 1, 4) cycles. Among women who slept <6 hours, 56.5% became pregnant over 6 cycles of follow-up (n=26 of 46), whereas among those who slept 7-8 hours, 67.4% became pregnant (n=321 of 476), and among women who slept ≥9 hours, 60.5% became pregnant (n=81 of 134). Women who slept ≥9 hours had 20% reduced fecundability compared to 7-8 hours (FOR: 0.80, 95% CI: 0.60, 1.07; Table 2.3). When we restricted to women trying to become pregnant for ≤3 cycles before study entry, the magnitude of association for sleep duration ≥9 (vs. 7-8 hours FOR: 0.70, 95% CI: 0.51, 0.97; Table 2.4) and fecundability became stronger. Sensitivity analyses excluding shift workers also showed stronger magnitude of association for sleep ≥9 hours (vs. 7-8 hours FOR: 0.64, 95% CI: 0.43, 0.96; Table 2.4) and lower fecundability as well.

Over 30% of women were rotating (n=207 of 1180, 17.5%) or night (n=275 of 1178, 23.3%) shift workers. Rotating shift work was not associated with fecundability, but night shift work was weakly associated with higher fecundability (vs. non-rotating shift work FOR: 1.07, 95% CI: 0.86, 1.32; vs. non-night shift work FOR: 1.17, 95% CI: 0.96, 1.42; Table 2.3). When evaluating the association between sleep midpoints and fecundability, later sleep midpoints (3rd tertile vs. 2nd tertile; FOR: 0.85, 95% CI: 0.69, 1.04; Table 2.3) were weakly associated with lower fecundability. Per hour increases in sleep midpoint were not associated with fecundability. Social jet lag was also weakly associated with lower fecundability (FOR: 0.92, 95% CI: 0.86, 1.00, per hour increase; Table 2.3), but not in categories of social jet lag. Restricting to women trying to become pregnant for ≤3 cycles before study entry resulted in the association for social jet lag and lower fecundability (FOR: 0.90, 95% CI: 0.82, 0.98, per hour increase) remaining similar (Table 2.4). Similar patterns were observed for night shift work (vs. non-night shift work; FOR: 1.13, 95% CI: 0.91, 1.41), rotating shift work (vs. non-rotating shift work; FOR: 1.07, 95% CI: 0.84, 1.38), and later sleep midpoints (3rd tertile vs. 2nd tertile; FOR: 0.85, 95% CI: 0.68, 1.06) and fecundability. Sleep midpoints on the continuous scale however were significantly associated with lower fecundability (per hour increase; FOR: 0.93, 95% CI: 0.86, 0.99). Excluding shift workers at baseline did not substantially change

associations between sleep midpoints and fecundability (3rd tertile vs. 2nd tertile: FOR: 0.86, 95% CI: 0.68, 1.09; Table 2.4), but attenuated associations between social jet lag and fecundability (FOR: 0.98, 95% CI: 0.88, 1.10, per hour increase).

Discussion

Among pregnancy planning women with a history of pregnancy loss, we observed that sleep duration ≥9 hours was potentially associated with reduced fecundability and that later sleep midpoints and greater social jet lag were also associated with weak reductions in fecundability. Overall, our findings weakly suggest a role of multiple sleep characteristics, particularly sleep duration and timing, in fecundability among reproductive aged women.

Our finding that sleep duration ≥9 hours was associated with a weak reduction in fecundability is consistent with findings from an in-vitro fertilization (IVF) cohort (105). Yet, our findings contrast those from PRESTO, which found that sleep <6 vs. 8 hours was weakly associated with reduced fecundability, but longer sleep durations were not (94). Sleep duration 4-6 hours was also associated with reduced probability of pregnancy vs. 7-8 hours sleep duration in the IVF cohort (105). In EAGeR, sleep duration <6 hours was not associated with fecundability and may be due to small numbers of women sleeping <6 hours. Our results suggest sleep duration ≥9 hours may be associated with reduced fecundability and together with prior studies suggest that perhaps both shorter and longer sleep durations may be weakly associated with reductions in fecundability (94, 105).

We found that night shift work, but not rotating shift work, was associated with a weak increase in fecundability suggesting that women in night shift work took less time to become pregnant compared to non-night shift workers. This finding may be explained by the fact that shift work was captured for the most recent job in EAGeR. It is possible some women may have shifted professions over preconception trying time. Unfortunately, we did not capture whether women recently changed jobs. In considering the literature on shift work and fecundability, PRESTO, a prospective study of shift work and TTP, reported a null association (94). In contrast, studies using retrospective recall of TTP or studies limited to shift workers who achieved pregnancy have reported reduced fecundability (95-100), though these study designs have been shown to be biased when estimating fecundability (106, 107). However, measures of shift work only capture circadian disruption among a shift working population and there is a need for future work to

consider the role of sleep timing as well. Importantly, our work extends on prior studies of shift work and fecundability through characterizing sleep timing using sleep midpoints as a proxy of chronotype.

We observed a marginal association for later sleep midpoints with reduced fecundability based on tertiles, and a weak, but significant association between per hour increases in sleep midpoints and reduced fecundability among women with ≤3 previous cycles of attempts. Together, both suggest there may be a potential weak association between later sleep midpoints and lower fecundability. One cross-sectional study examined chronotype and participants' self-reported pregnancy history and suggested that intermediate chronotypes compared to morning chronotypes reported greater difficulty becoming pregnant (67). However, this study assessed prior difficulties in becoming pregnant and is not directly comparable to studies that measure fecundability. Beyond chronotype, we also report a weak, suggestive reduction in fecundability for increasing hours of social jet lag, but not in individual categories of social jet lag. This finding may suggest that mild circadian disruption through having different sleep timing between weekdays and weekends may be associated with delayed fecundability. While social jet lag has not been directly evaluated with fecundability, social jet lag has been associated with obesity, depression, and menstrual symptom severity, and thus it is plausible that social jet lag may be associated with reduced fecundability via these conditions (6, 39, 59). With few studies characterizing sleep midpoint, social jet lag, and fecundability, further research using objective sleep timing assessments is needed to clarify the role of these factors with fecundability.

Sleep and fecundability may be linked through biological pathways in which sleep behaviors influence the sleep-wake cycle. The sleep-wake cycle involves melatonin, which regulates sleep timing (43, 85, 86, 108-111). Melatonin may also play a role in gonadotropin releasing hormone (GnRH) regulation and steroidogenesis, which are involved in ovulation and blastocyst implantation (22, 23, 25, 26). Therefore, sleep may influence melatonin and thus fecundability through alteration of reproductive hormones, ovulation, and implantation. Endogenous circadian rhythms and molecular clocks may also relate sleep to fecundability as evidence from animal models has shown that clock genes play a role in implantation and pregnancy establishment (29, 112). Social jet lag effects from differences in sleep timing between weekends vs. weekdays may result in misalignment of biological rhythms and altered clock gene expression (68, 69). Clock genes also vary among chronotypes, and later chronotypes have been associated

with longer menstrual cycles and shifts in the timing of hormone peaks during the menstrual cycle (38, 67). This may suggest that different chronotypes may have different underlying risks for menstrual cycle dysfunction and could influence fecundability.

Strengths and Limitations

Our study has many strengths including longitudinal assessment of pregnancy attempts, high sensitivity β-hCG testing to capture both recognized and unrecognized pregnancies, assessment of multiple dimensions of sleep, and minimal participant dropout. A limitation in our study was that assessment of sleep duration, timing, and shift work was limited to baseline self-report; other objective assessments such as actigraphy, may provide a more robust assessment. However, we expect our sleep characteristics and shift work to be non-differentially misclassified with respect to fecundability, given that sleep and shift work were measured prior to pregnancy. Our measures of sleep midpoint and social jet lag were based on calculations using the sleep midpoints of weekend and weekday sleep and assuming that weekend sleep midpoints represent the midpoints of days "free" from work or the influence of an alarm clock (4, 6). Unfortunately, we did not have information on work or school schedules to determine whether weekends were in fact "free days." In addition, our measurement of rotating and night shift work was based on participant self-report of most recent job with rotating or night shift work and may not reflect their current work schedule. Further, we only assessed baseline, usual sleep characteristics and cannot examine the longitudinal impacts of sleep characteristics in relation to reproductive outcomes. Lastly, the EAGeR population was comprised of mostly healthy, white women attempting pregnancy who had a history of pregnancy loss, which may limit our generalizability. Though, almost 30% of reproductive age women have experienced a prior pregnancy loss and thus represent a large proportion of women to whom these results would be generalizable (83). Future research among diverse populations is warranted.

Conclusions

Our results suggest that sleep duration ≥9 hours, later sleep midpoints, and greater social jet lag may be associated with reduced fecundability. This work on sleep characteristics and fecundability extends on prior work in this field by broadening the context of sleep by highlighting the need to evaluate sleep timing. Future research on sleep and fecundability should incorporate multiple domains of sleep and use

both validated subjective scales and objective measures of sleep to better characterize the nuanced role of sleep with fecundability.

CHAPTER 3

PRECONCEPTION SLEEP, LIVE BIRTH, PREGNANCY LOSS, AND ADVERSE PREGNANCY OUTCOMES

Introduction: Live Birth

Infertility is widespread with nearly 15% of couples unable to become pregnant after 12 months of trying to conceive (113, 114). In any potential cycle of conception, only 24-28% of women not currently on contraception successfully conceive; however, it is estimated that nearly 1/3rd of conceptuses will be lost (115-117). Therefore, conceiving and carrying a pregnancy to birth remains challenging for many couples with some choosing to utilize infertility treatments to improve chances of a live born infant (118). Yet, few states mandate coverage for fertility treatments and they unfortunately remain cost-prohibitive and inequitable (119-122). Prior research has examined lifestyle factors among pregnancy planning and infertile couples seeking fertility treatments to try to improve fertility and successful delivery of a live born infant (52, 123, 124). Sleep is increasingly being considered as one possible influence on fertility (24).

Sleep may be an important modifiable lifestyle factor for live birth given its potential influence on many steps in the process of successful human reproduction throughout the *periconceptional period* (i.e., both *preconception* and *pregnancy periods*) (125). Studies in both animals and humans have noted a possible role of sleep in regulating the circadian rhythms of reproductive hormones that select an oocyte for ovulation and prepare the uterine lining for implantation (22, 23, 25, 26, 28-31, 62). Beyond the role of sleep in the biological rhythms of reproductive hormones, intermediates of the sleep-wake cycle (melatonin and clock genes), play a role in embryo implantation at pregnancy establishment and in early gestation through mechanisms in placental development and decidualization (22, 23, 25, 26, 29, 68, 69, 85, 86, 108, 109, 112, 126-129). We hypothesize that usual sleep during the *preconception period* may influence embryological development and placental formation in an early critical window of gestation, or may serve as a proxy of sleep within this critical development window (130-132). This critical and sensitive window near the time of conception, embryological development, and early placental formation may represent an important timepoint in which *preconception* sleep may influence maternal-fetal health and probability of live birth (125, 133, 134). Given the potential for preconception sleep to influence many events in human

reproduction, it is important to evaluate sleep in the preconception period to better understand the role of sleep and live birth in this critical window of development.

When considering epidemiological evidence of sleep, fertility and live birth, there is evidence to suggest shorter sleep duration and night shift work are associated with longer time-to-pregnancy (94, 95). However, we are aware of no studies that have explicitly evaluated sleep duration or sleep timing and the probability of live birth, though a single study has evaluated *periconception and pregnancy* shift work and live birth (135). This retrospective study of 440 women who worked continuously in a semiconductor factory for more than 10 years reported that women in consistent day shifts throughout *periconception* had greater odds of live birth (OR: 1.7, 95% CI: 1.0, 3.0) compared to women in shift work (135). This study unfortunately did not differentiate between preconception and pregnancy shift work, but may preliminarily suggest that circadian disruption through shift work may lower probability of live birth, though additional work is needed to understand whether other relevant sleep domains including sleep duration and sleep timing may also be related to live birth.

Therefore, our aim was to evaluate the role of *preconception* sleep in live birth. Many reproductive aged women in the US do not meet recommended sleep guidelines (9, 101). Given 50-60% of pregnancies are planned and for the potential of poor sleep to delay conception and contribute to infertility, this work may illuminate potentially modifiable factors, such as sleep, to improve likelihood of live birth (133, 136). Furthermore, evaluating sleep as early as the *preconception period* may help identify the role of sleep in an early and sensitive window that can impact pregnancy throughout gestation.

Introduction: Pregnancy Loss and Adverse Pregnancy Outcomes

Pregnancy loss and adverse pregnancy outcomes affect a stark number of pregnancies. Up to 30% of pregnancies end in a loss, and of those not lost approximately 15-20% are affected by preterm birth, hypertensive disorders of pregnancy, or gestational diabetes (GDM) (83, 137-140). Adverse pregnancy outcomes may also increase risk to adverse maternal health (141-144), as well as increase risk of offspring developmental delays (145-147). Given these high incidences, there is a need to identify modifiable risk factors for these outcomes. Sleep may hold value as one such modifiable risk factor. Sleep behaviors influence intermediates of the sleep-wake cycle that are involved in many reproductive processes

throughout the *periconception period* (24, 125). Therefore, it may be important to understand how sleep may be related to pregnancy loss and adverse pregnancy outcomes throughout periconception.

Evidence from population-based studies of mid-pregnancy sleep, pregnancy loss, and adverse pregnancy outcomes generally support a role for mid-pregnancy sleep and risk of these adverse outcomes (58, 148-158). Sleep duration ≤8 hours (148) and shift work (149-152) in mid-pregnancy are associated with greater risk of pregnancy loss in the late first and early second trimesters. Literature evaluating mid-pregnancy sleep also provides evidence that poor sleep throughout pregnancy may be associated with greater risk of preterm birth, hypertensive disorders of pregnancy, and GDM (58, 153-158). However, there are two significant gaps in the literature evaluating these outcomes that center on the lack of studies that have considered *sleep during the preconception period*. First, most studies of pregnancy loss recruited women during their pregnancy near late first trimester (148-152). However, the majority of pregnancy losses (~80%) occur well before this timepoint and before women recognize they are pregnant (117, 159). Thus, many studies have not evaluated these early, implantation failures that occur as early as 3 weeks gestation, which may be related to the influence of *preconception sleep* on early embryo and placental development at conception and in the first weeks of pregnancy establishment (117, 130, 131, 160, 161). Second, the preconception period may serve as a critical exposure window during which poor sleep could influence the physiology of the endometrial lining and early placental tissues (125, 133, 134). These disruptions may have consequences on adverse outcomes in pregnancy. While many studies have evaluated acute effects of mid-pregnancy sleep on adverse pregnancy outcomes, we are not aware of any studies that have <u>prospectively</u> evaluated *preconception sleep and risk of adverse pregnancy outcomes.* Evaluating preconception sleep in risk of adverse pregnancy outcomes may highlight the *preconception period* as a sensitive window for sleep behaviors.

The sleep-wake cycle is related to many functions throughout human reproduction. Importantly, sleep during the preconception period influences the circadian rhythms of reproductive hormones, which impacts the development of the uterine lining, pregnancy establishment and maintenance (22, 23, 25-31). Past the preconception period, we hypothesize that preconception sleep may still serve as a proxy of usual sleep in early pregnancy (3-5 weeks gestation) when developmental changes begin to occur within a dividing embryo and within early structures that will differentiate into the placenta (130-132). This window

of conception and early placental development is an important timepoint in which preconception sleep may influence this sensitive process of development (125, 133, 134). Mid-pregnancy sleep may also influence risk of adverse pregnancy outcomes (58, 153-158). However, it is possible these associations may be driven by reverse causation and disruptions to sleep during earlier critical windows of development that may lead to both changes in sleep patterns and adverse pregnancy outcomes.

In considering the potential impact of sleep on important placental physiology, and the impact this may have on pregnancy maintenance and health, there is a need for studies aimed at improving our understanding of *preconception sleep* in pregnancy loss and adverse pregnancy outcomes (130-132). To address this important research gap, a second aim of this chapter was to examine how preconception sleep is associated with pregnancy loss and adverse pregnancy outcomes..

Methods

Participants

This is a secondary analysis of a cohort nested within the EAGeR trial conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) to examine the effect of low-dose aspirin on live birth and pregnancy loss (40, 41). Inclusion and exclusion criteria and full details for the EAGeR study are previously described. Briefly, EAGeR (n=1228) was a block-randomized, double-blind, placebo controlled trial conducted in the US including women recruited from the states of Utah, New York, Pennsylvania, and Colorado from 2006-2012. Women must have had 1-2 prior pregnancy losses, have an intact uterus, tubes, and ovaries, be aged 18-40 at baseline, and have regular menstrual cycles (21-42 days length). Women must also not have been pregnant at baseline, and be actively trying to conceive. Women were excluded if they had contraindications for NSAIDs, presence of major medical disorders, history of infertility, or were undergoing assistive reproductive therapies. Women were followed for up to 6 cycles while trying to become pregnant and throughout pregnancy for those who became pregnant (n=797). Fertility monitors (ClearBlue Easy; Inverness Medical) were used during the preconception cycles to improve timing of intercourse and to optimize conception, and to aid in clinic visit scheduling (41).

Assessment of Sleep Characteristics

Sleep characteristics including usual, average bedtime, wake time, and sleep onset latency were measured at baseline via self-report for both weekdays and weekends. Using these self-reported characteristics we calculated: 1) sleep duration, 2) sleep-debt corrected midpoint of sleep on "free" days, and 3) social jet lag. Sleep duration was calculated as the difference in usual time to get up and bedtime and subtracting the amount of usual time to fall asleep for weekdays and weekends. Sleep duration was weighted for weekday (5/7) and weekend (2/7) contributions. We evaluated two sleep timing measures: sleep midpoint and social jet lag. We calculated sleep midpoint as the midpoint of an average weekend sleep interval (the midpoint between sleep onset and waking) and subtracting half the difference between weekend and total sleep durations in order to correct for sleep debt accrued over weekdays (3, 42, 43). Specifically, sleep debt is assumed to be accrued over weekdays and may be greater for individuals with greater tendency to be evening persons (later sleep midpoints) because typical workdays would impact their need for sleep on the weekends. This sleep debt is compensated for greater "free day" sleep on weekends assuming they are free from the influence of an alarm clock. Thus, correcting sleep midpoints for sleep debt provides a better proxy of chronotype, the biological construct of "temporal phenotype" (morning type or evening type), than weighting sleep midpoint for weekdays and weekends (3, 42, 43). We applied the sleep debt correction to sleep midpoint given the cohort included both employed and unemployed women and the inability to distinguish when individuals are free from work or other responsibilities that may impact sleep midpoint. For individuals who sleep longer on weekdays than weekends, we did not correct their weekend sleep midpoints for sleep debt (3). We also evaluated social jet lag, or the discrepancy in usual sleep timing between work days and "free" days (4). This was accomplished by taking the absolute difference between weekend and weekday sleep midpoints and comparing this in categories <1, 1-2, and ≥ 2 hours as has been done in prior literature (3, 4, 6). In calculating social jet lag, we assume that weekends were "free" days, or days in which individuals were free from work and the influence of an alarm clock, as we did not have detailed information on work schedules (4, 6). Shift work was assessed at baseline by asking if participants had night shift work and rotating shift work schedules for their most recent job (current or past employment). Additional details on sleep variable assessment and operationalization are shown in Table 3.1.

Assessment of Pregnancy Loss, Live Birth, and Adverse Pregnancy Outcomes

Live birth, pregnancy loss, preterm birth, hypertensive disorders of pregnancy, and GDM ascertainment have been described previously in detail (40, 161). In brief, pregnancies were ascertained via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses and were augmented with daily first-morning urine collected over the first two cycles of follow-up and spot urine pregnancy tests at monthly clinic visits. HCG-detected pregnancy loss was defined as 1) positive urine pregnancy tests followed by absence of clinical signs of pregnancy at 6-7 weeks' ultrasound, or 2) positive urine hCG test from batched augmented assays followed by absence of pregnancy from positive tests conducted at home or in clinic (123). Clinically-defined pregnancy losses were losses that were observed by the participant or the physician and occurred after the clinical ultrasound.

Date of delivery, live birth, and adverse pregnancy outcomes such as hypertensive disorders of pregnancy (including preeclampsia), and GDM were obtained by postpartum phone interview and medical chart abstraction (161-163). Gestational age was calculated based on the 6-7 week clinical visit ultrasounds for most pregnancies and if ultrasounds were not available, gestational age was calculated based on last menstrual period determined using home based fertility monitors provided by the study (41, 163). Preterm birth was defined as any birth between 20 weeks and 0 days and 36 weeks and 6 days' gestation (161, 163).

Assessment of Covariates

Participants completed baseline questionnaires assessing demographics, lifestyle, substance use, and reproductive and familial medical history. Weight (kg) and height (m) were measured using standardized protocols and were used to calculate BMI. Smoking status was measured via baseline questionnaire and urine cotinine biomarkers. Sleep aid, and melatonin supplement use over the past 12 months were assessed at baseline via self-report. Marijuana, opioid, and antidepressant metabolites were measured from urine samples collected at baseline. Urine samples were assayed using the Drug of Abuse IV Ultra chemiluminescent immunoassay measured on the Evidence Investigator (Randox Toxicology, County Antrim, United Kingdom). Positive screens were indicated based on standard manufacture-based cutoffs.

Statistical Analysis

At baseline, most women in EAGeR (99.3%, n=1220/1228) reported sleep data with only eight women missing data for usual bedtime or waking time (<1.0%, n=8/1228). As such, we summarized participant characteristics and sociodemographics by categories of preconception sleep duration and preconception sleep midpoint among women with complete sleep data (n=1220). We used log-Poisson models to estimate relative risks (RR) and 95% confidence intervals (CIs) for associations between preconception sleep characteristics, live birth, pregnancy loss, and adverse pregnancy outcomes. For associations with live birth, sleep duration was examined as a 5-level categorical variable (hours; <6, 6-7, 7-8 (referent), 8-9, and ≥9) to investigate associations at shorter (<6 hours) and longer (≥9 hours) durations. Due to constraints on sample size in analyses of pregnancy loss and adverse pregnancy outcomes, we examined sleep duration as a 3-level categorical variable (sleep duration (hours; <7, 7-9 (referent), and ≥9)) in which 7-9 hours was chosen as the referent category given this is the recommended amount of sleep for adults aged 18-64 years (164, 165). Sleep midpoint was analyzed as tertiles (tertiles; tertile 1, tertile 2 (referent), tertile 3) in analyses of live birth, pregnancy loss, and adverse pregnancy outcomes given the lack of population-appropriate cut points for early, normal, and late sleep midpoints for reproductive aged women. For associations of live birth, social jet lag was analyzed as hourly categorical levels (hours; 0-1 (referent), 1-2, ≥2) based on prior work indicating associations within hour-levels of social jet lag with adverse health outcomes and reproductive health (6, 39). We concatenated social jet lag 1-2 hours and ≥2 hours categories into a single ≥1 hours social jet lag category for analyses of pregnancy loss and adverse pregnancy outcomes due to constraints on power. Linear tests for trend were conducted evaluating sleep duration, sleep midpoint, and social jet lag in association with live birth, pregnancy loss and adverse pregnancy outcomes. Sleep duration, sleep midpoint and social jet lag were not linearly related to live birth, or pregnancy loss. Only social jet lag (p=0.02) was linearly related to the composite adverse pregnancy outcome and as such, continuous models are only presented for social jet lag and the composite outcome. Rotating shift work (vs. non-rotating shift work) and night shift work (vs. non-night shift work) were assessed as binary variables. For pregnancy loss analyses, we also stratified by pregnancy loss type (hCG-defined vs. clinical) to determine whether there are differences in the association between preconception sleep and pregnancy loss by early first trimester hCG pregnancy losses, and mid-late, first trimester and early second trimester losses. We also hypothesized that preterm birth, hypertensive disorders of

pregnancy, and GDM are related to preconception sleep characteristics through early gestational influences on uterine implantation mechanisms and placental formation that begins as early as 3 weeks gestation (130-132). Therefore, in addition to analyzing outcomes individually, we aggregated preterm birth, hypertensive disorders of pregnancy, and GDM together as a composite outcome representing adverse pregnancy outcomes of placental origin to explore this hypothesis. Live birth, pregnancy loss, and adverse pregnancy outcomes models were adjusted for *a priori* selected confounders including age, BMI, parity, stress, opioid use, marijuana use, antidepressant use, race, education, smoking, employment, alcohol, caffeine, season, sleep aid use, and exercise. EAGeR was originally conducted as a randomized control trial with half of participants randomized to receive low dose aspirin (81 mg) and folic acid (400 mcg) and the other half of participants randomized to receive a placebo and folic acid (400 mcg) (40, 41). Treatment arm assignment was not associated with sleep characteristics, and thus was not considered a confounder in our associations.

Inverse Probability Weights to Account for Conditional Probabilities Across Reproduction

Our models evaluating probability of live birth, risk of pregnancy loss, and risk of adverse pregnancy outcomes are based on multiple conditional probabilities including: 1) remaining in the cohort, 2) becoming pregnant, and 3) remaining pregnant ≥20 weeks gestation (166, 167). Sleep behaviors during the preconception period may influence early withdrawal from the cohort, the probability of pregnancy, and remaining pregnant ≥20 weeks gestation. This may give rise to selection bias when evaluating associations of preconception sleep, live birth, pregnancy loss, and adverse pregnancy outcomes given sleep may be related to the outcome of interest as well as the probability of remaining in the cohort or surviving long enough to have the outcome of interest (166). Throughout these analyses, we used stabilized inverse probability weights to account for potential selection biases at each conditional probability as relevant for a given analysis (168). Given we are interested in evaluating the probability of live birth among all women who enrolled in EAGeR, we evaluated each sleep characteristic in association with live birth among all participants (n=1228). All models of live birth, pregnancy loss, and adverse pregnancy outcomes included stabilized inverse probability weights to account for early withdrawal (n=140/1228) over follow-up in EAGeR, which may have been influenced by sleep characteristics. We generated these stabilized inverse probability weights to account for potential selection bias from early withdrawal using factors including

sleep characteristics, age, BMI, history of previous pregnancy losses, number of live births, treatment group, marital status, and parity.

For analyses of pregnancy loss, we conditioned on pregnancy status given that women were not at risk of pregnancy loss unless they became pregnant (n=797/1228). Sleep characteristics may have influenced the probability of pregnancy. Thus, we used inverse probability of pregnancy weights (IPPW) to account for potential selection biases due to conditioning on pregnancy in these analyses and given that sleep may have influenced this conditional probability (161, 166, 168, 169). IPPW were generated for pregnancy loss and hCG pregnancy losses based on the conditional probability of becoming pregnant (n=797) whereas for clinical pregnancy losses, we additionally generated IPPW based on the probability of the pregnancy surviving to clinical recognition (n=732). IPPW were generated using models predicting associations with pregnancy including factors such as sleep characteristics, treatment group, parity, marital status, age, and number of previous losses (170). Given the probability of pregnancy is also conditional on remaining in the cohort and being observed, we incorporated the early withdrawal weights by cross-multiplying them with IPPW. Cross-multiplication of weights has been recommended in prior studies using multiple weights to account for potential selection biases from conditioning on multiple probabilistic events (167).

Lastly, in analyses of adverse pregnancy outcomes, we were interested in estimating the risk of adverse pregnancy outcomes among women with pregnancies lasting ≥20 weeks gestation (n=598). Pregnancies must have lasted until at least 20 weeks gestation in order to be at risk of preterm birth, a hypertensive disorder of pregnancy, or GDM given this gestational age is the minimum time by which we defined preterm birth and when screening for adverse pregnancy outcomes would begin (161, 163). We also used stabilized inverse probability of pregnancy weights to account for potential selection biases from conditioning on pregnancies lasting ≥20 weeks gestation given that sleep may have also influenced this conditional probability. These weights were generated using factors including sleep characteristics, treatment group, parity, marital status, age, and number of previous losses (170). Furthermore, given that pregnancies lasting at least 20 weeks gestation were conditional upon women having become pregnant and having remained in the cohort to be observed over follow-up, we also cross-multiplied these weights with IPPW and weights to account for early withdrawal as has been done in prior literature (167).

We used multiple imputation to address missing exposure and covariate information using n=10 datasets and 20 burn-in iterations (48). Thus, our crude and multivariate adjusted analyses are conducted using information from the full cohort (n=1228). However, for our analyses we restrict to women who completed follow-up (n=1088) for live birth, to pregnant women (n=797) for pregnancy loss, and on pregnancies that lasted ≥20 weeks gestation (n=598) for adverse pregnancy outcomes and we interpret our findings among the full cohort. Statistical analyses were conducted using SAS v 9.4 software (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics

As shown in Table 3.2, comparing participants by categories of sleep duration, 7-8 hours was the most frequent sleep duration group (38.8%) with slightly fewer women sleeping 8-9 hours (35.2%). Compared to women who slept 7-8 hours, women who slept ≥9 hours were younger, had lower BMI, and lower education. These women were also more likely to use opioids, marijuana, antidepressants, and sleep aids compared to women with 7-8 hours sleep duration. Women who slept <6 hours (vs. 7-8 hours) were also more likely to have children. Women who slept <6 hours or ≥9 hours were more likely to work as night and rotating shift workers, were more likely to withdraw from the study, were less likely to be married, and were more likely to smoke compared to women who slept 7-8 hours. The majority of women tended to have sleep midpoints <5:00AM with the medians of sleep midpoint tertiles being: 2:45AM (IQR: 2:20AM-3:00AM) for tertile 1, 3:36AM (IQR: 3:25AM-3:48AM) for tertile 2, and 4:40AM (IQR: 4:21AM-5:17AM) for tertile 3 (Supplementary Table 3.1S). Women with sleep midpoints in the 1st and 2nd tertiles of the distribution tended to be older, have lower social jet lag, and were less likely to use opioids and marijuana, but have greater education, and parity compared to women with sleep midpoints in the 3rd tertile (Supplementary Table 3.1S). In considering social jet lag and the timing of sleep, 3.0% (n=36) slept later on weekdays than weekends, 70.9% (n=865) slept later on weekends than weekdays and 26.1% (n=319) had the same timing of sleep on weekdays and weekends.

Live Birth

Among n=1228 women, n=797 women became pregnant, and n=597 delivered live births at the end of follow-up. We examined associations between preconception measures of sleep duration, shift work,

and sleep timing with probability of live birth (Table 3.3). In multivariate models controlling for confounding, preconception sleep duration (vs. 7-8 hours, <6 hours RR: 0.98, 95% CI: 0.61, 1.57; 6-7 hours RR: 0.99, 95% CI: 0.75, 1.32; 8-9 hours RR: 0.89, 0.74, 1.07; ≥9 hours RR: 0.89, 95% CI: 0.66, 1.19) was not associated with the probability of live birth. Similar results were observed for rotating shift work (vs. non-rotating shift work RR: 1.05, 95% CI: 0.85, 1.29), night shift work (vs. non-night shift work RR: 1.07, 95% CI: 0.88, 1.30), sleep midpoint (vs. tertile 2; tertile 1 RR: 1.03, 95% CI: 0.85, 1.26; tertile 3 RR: 0.92, 95% CI: 0.75, 1.13), and social jet lag (vs. 0-1 hours; 1-2 hours RR: 1.05, 95% CI: 0.86, 1.29; ≥2 hours RR: 0.99, 95% CI: 0.72, 1.35) in relation to the probability of live birth.

Pregnancy Loss

Overall, 23.6% (188/797) of pregnant women in EAGeR experienced a pregnancy loss of which, 29.3% (55/188) were hCG pregnancy losses occurring at a median 4 weeks gestation (Quartile (Q)1: 3 Q3: 5) and 70.7% (133/188) were clinically recognized losses occurring at a median 8.7 weeks gestation (Q1: 7.7, Q3: 10.9). After adjustment for confounders, preconception sleep duration <7 hours (vs. 7-9 hours RR: 0.98, 95% CI: 0.64, 1.48) and ≥9 hours (vs. 7-9 hours RR: 1.24, 95% CI: 0.80, 1.94) were not associated with risk of pregnancy loss (Table 3.4). Similar null associations were observed for rotating shift work (vs. non-rotating shift work RR: 0.99, 95% CI: 0.67, 1.46), night shift work (vs. non-night shift work RR: 0.99, 95% CI: 0.70, 1.41), sleep midpoints (vs. tertile 2; tertile 1 RR: 0.98, 95% CI: 0.69, 1.40; tertile 3 RR: 1.06, 95% CI: 0.74, 1.51), and social jet lag (vs. 0-1 hours; ≥1 hours RR: 0.93, 95% CI: 0.68, 1.28; Table 3.4). When we stratified analyses by risk of hCG-defined and clinically recognized pregnancy losses, associations remained similar (Supplementary Tables 3.2S, 3.3S).

Adverse Pregnancy Outcomes

Overall, 19.7% (118/598) of women who were pregnant at 20 weeks gestation had an adverse pregnancy outcome. Of the 118 women who experienced an adverse outcome, 44.1% (52/118) delivered a preterm birth, 52.5% (62/118) experienced hypertensive disorders of pregnancy, and 18.6% (22/118) developed GDM. Few women (15.3%; 18/118) experienced multiple adverse pregnancy outcomes.

Sleep duration was not associated with risk of the composite outcome (<7 vs. 7-9 hours; RR: 1.28, 95% CI: 0.76, 2.13; ≥9 vs. 7-9 hours; RR: 1.06, 95% CI: 0.56, 2.00; Table 3.5). When evaluating each outcome (preterm birth, hypertensive disorders of pregnancy, and GDM) separately, sleep duration was not

associated with GDM or hypertensive disorders of pregnancy, but sleep duration <7 hours was associated with greater risk of preterm birth (<7 vs. 7-9 hours; RR: 2.64, 95% CI: 1.35, 5.17; Supplementary Table 3.4S).

Neither rotating nor night shift work was associated with adverse pregnancy outcomes as a composite outcome (Table 3.5) or in analyses considering outcomes individually (Supplementary Table 3.4S). When considering sleep timing measures, earlier, but not later sleep midpoints (1st tertile vs. 2nd tertile; RR: 0.62, 95% CI: 0.39, 0.99; 3rd tertile vs. 2nd tertile; RR: 0.98, 95% CI: 0.64, 1.52) were associated with reduced risk of the composite outcome. When evaluating individual outcomes, associations between earlier midpoints and preterm birth (1st tertile vs. 2nd tertile; RR: 0.49, 95% CI: 0.24, 1.00), hypertensive disorders of pregnancy (1st tertile vs. 2nd tertile; RR: 0.66, 95% CI: 0.35, 1.26), and GDM (1st tertile vs. 2nd tertile; RR: 0.44, 95% CI: 0.14, 1.37) were similarly associated (Supplementary Table 3.4S). Social jet lag was significantly associated with greater risk of the composite outcome (≥1 vs. <1 hour; RR: 1.60, 95% CI: 1.06, 2.40; Table 3.5), but not as a per hour increase in social jet lag (per hour; RR: 1.01, 0.86, 1.18; Table 3.5). Evaluating each adverse outcome individually (Supplementary Table 3.4S) also showed similar associations between social jet lag and hypertensive disorders of pregnancy (≥1 vs. <1 hour; RR: 1.68, 95% CI: 0.96, 2.95), and GDM (≥1 vs. <1 hour; RR: 3.63, 95% CI: 1.23, 10.7), but not preterm birth (≥1 vs. <1 hour; RR: 1.01, 95% CI: 0.55, 1.85).

Discussion

In this prospective cohort of pregnancy planning women with a history of pregnancy loss, *preconception* sleep duration, sleep timing, and shift work were not associated with probability of live birth or risk of pregnancy loss. Further, stratifying models by early, hCG-defined, and later gestation, clinically recognized pregnancy losses resulted in similar findings. Preconception measures of sleep duration and shift work were not associated with our composite of adverse pregnancy outcomes either. However, both sleep timing measures (sleep midpoint and social jet lag) were associated with the composite adverse pregnancy outcome. First, women who had early, preconception sleep midpoints (1st vs. 2nd tertile) had 38% reduced risk of the composite outcome. This association persisted among each individual outcome, but was imprecise. Second, women with ≥1 hour (vs. <1 hour) social jet lag had almost 60% greater risk of the composite adverse pregnancy outcome. In analyses of individual outcomes, this association appeared to

be related to hypertensive disorders of pregnancy, and potentially GDM. When evaluated among each adverse pregnancy outcome, sleep duration <7 hours during preconception was associated with a 2.44 fold increased risk of preterm birth.

In totality, these results may preliminarily suggest that sleep duration, sleep midpoint, and social jet lag during the preconception period may be associated with risk of adverse pregnancy outcomes. However, preconception sleep was not associated with live birth or pregnancy loss. Our findings are consistent with studies of mid-pregnancy sleep and suggestively expand upon these studies by weakly suggesting that sleep during the preconception period may potentially be associated with outcomes in mid-pregnancy. In interpreting GDM-specific associations, we were unfortunately limited in power. While we present associations for sleep and GDM in the results section and in Table 3.4S, and interpret it in the context of the composite outcome, we cannot adequately interpret the GDM-specific findings in the context of prior literature given the low case incidence. Even though our findings for early preconception social jet lag with GDM may suggest an association, these findings should be considered with caution.

Preconception Sleep and Probability of Live Birth

Sleep during the preconception period was not associated with live birth. We are aware of only one other study that has explicitly evaluated sleep duration, sleep midpoint, social jet lag, or shift work in association with live birth (135). Lin et al. reported in a retrospective cohort of daytime and night shift workers that women in consistent daytime positions throughout preconception and pregnancy were more likely to have had a live birth compared to women in shift work (OR: 1.7, 95% CI: 1.0-3.0) (135). Shift workers in this study worked 12-hour shifts of 6 day shifts-3 rest days or 6 night shifts-3 rest days and may have had stronger circadian disruption from a cumulative average effect of night shift work compared to women in EAGeR. Shift work in EAGeR was reported for the most recent job (not current). Women may have also changed their profession at pregnancy. Unfortunately, we did not collect mid-pregnancy shift work in EAGeR, and it is possible women in EAGeR may not have had the same shift working job during pregnancy. Therefore, in totality there may be an association of preconception and periconception shift work and lower probability of live birth. However, there is a need for additional studies to evaluate the probability of live birth among shift workers throughout the preconception and pregnancy periods to identify if there are cumulative average, usual, or acute effects of shift work on live birth.

To our knowledge, no studies have evaluated sleep duration, sleep midpoint, or social jet lag and probability of live birth. Studies to date have noted that preconception shorter sleep duration, and shift work may result in lower fecundability among pregnancy planning women (94-100). Further, a systematic review of sleep and studies of women seeking IVF treatments noted that both short and long sleep duration and shift work was associated with poor pregnancy rates, and poor oocyte retrieval, respectively (171). While there is evidence for a role of poor sleep in longer time-to-pregnancy and poor IVF outcomes, studies have yet to follow women through delivery to determine if preconception sleep may impact live birth.

Our findings may suggest that preconception sleep is not associated with probability of live birth. However, given the biological plausibility for sleep to influence pregnancy viability as early as weeks 3-5 of gestation and the lack of studies on sleep and live birth, there is a need for additional studies of sleep and live birth (130-132). Future studies should examine sleep longitudinally throughout both the preconception and mid-pregnancy periods using objective measures. Evaluating sleep throughout periconception will help identify usual, acute, and cumulative average effects of sleep on live birth.

Preconception Sleep and Pregnancy Loss

Sleep duration during the preconception period was not associated with risk of pregnancy loss. We are not aware of other studies evaluating this association. However, findings from a case-control study suggest that sleep ≤8 hours/day in mid-pregnancy was associated with pregnancy loss in both the 1st trimester and 2nd trimesters (148). The differences in timing of our sleep assessments (preconception vs. mid-pregnancy) may potentially account for discrepancies between our findings. Importantly, we are one of the first to characterize associations of preconception sleep duration with early hCG pregnancy losses, including many unrecognized pregnancies occurring within the first 3-4 weeks of pregnancy. However, with few studies that have assessed sleep duration and risk of pregnancy loss, it is unclear if sleep duration in either time period may be related to risk of pregnancy loss. Further study is needed to evaluate the role of sleep duration throughout the periconceptional period in pregnancy loss using objective and longitudinal sleep measures to clarify these findings.

We observed no association between rotating shift work, or night shift work and risk of pregnancy loss and associations did not differ when stratified by hCG or clinically recognized pregnancy losses. Our

null results for shift work and hCG pregnancy losses generally agree with results reported in the Danish Working Hour Database study, which evaluated night shift work in the two weeks prior to miscarriage between 4-8 weeks gestation (150). In contrast, findings from studies of mid-pregnancy shift work have noted associations with 2nd trimester pregnancy losses and stillbirths (149-151). Together, these results suggest no association between night or rotating shift work with these early gestational, hCG pregnancy losses. This is also plausible given that embryological genetic anomalies may account for the majority of these early hCG losses rather than preconception sleep behaviors (172). However, this evidence may also suggest that mid-pregnancy shift work may still be associated with 2nd trimester pregnancy losses and stillbirths. Given the conflicting evidence of preconception and mid-pregnancy shift work with later gestation pregnancy losses, there is need for larger studies of shift work and pregnancy loss that follow women prospectively to better understand how shift work may influence pregnancy loss acutely and cumulatively throughout periconception.

To our knowledge, no population based studies have evaluated associations between preconception or mid-pregnancy sleep timing (sleep midpoints and social jet lag) and risk of pregnancy loss. In considering the current state of this literature, it is unclear how sleep timing or circadian disruption during the preconception period may be related to risk of pregnancy loss even though our results suggest there is no association. Further study is needed to evaluate both sleep midpoint and social jet lag throughout periconception to better understand the role of sleep timing in risk of pregnancy loss given the potential for sleep timing to influence pregnancy establishment and placental development in a critical period of early gestation.

Preconception Sleep Duration and Adverse Pregnancy Outcomes

We found no association between preconception sleep duration and risk of adverse pregnancy outcomes using our composite outcome measure. However, preconception sleep duration <7 hours was associated with risk of preterm birth. To our knowledge the Japan Environment and Children's Study (JECS) is the only other study that evaluated preconception sleep duration and risk of preterm birth (173). The JECS cohort (n=103,099) recruited women in their first trimester and reported that preconception sleep duration retrospectively recalled was not associated with risk of preterm birth (173). However, the reason for differences in our results may be due to non-negligible population differences in short sleep duration

prevalence (<7 hours sleep prevalence; EAGeR: 13.5% vs. JECS: 26.7%), which is higher compared to Western countries (173). Thus, the results from JECS may not be generalizable to Western populations. Our results agree with most literature of mid-pregnancy short sleep duration (<5 up to ≤7 hours) and preterm birth (174-177). Together with the literature of mid-pregnancy sleep duration, our results may weakly suggest that sleep duration ≤7 hours during the preconception period may also be related to risk of preterm birth in pregnancy. Given our results may suggest that sleep duration ≤7 hours may be associated with risk of preterm birth as early as the preconception period, further studies of sleep duration are needed to verify our associations with preterm birth.

Preconception sleep duration was not associated with risk of hypertensive disorders of pregnancy. No other studies have evaluated preconception sleep duration and risk of hypertensive disorders in pregnancy and studies using mid-pregnancy sleep remain sparse and inconsistent (58, 155, 157). However, findings from the large Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be (NuMoM2b) cohort (n=10038) may suggest there is an association between sleep duration <7 hours measured in the first trimester and risk of hypertensive disorders of pregnancy, but not with sleep duration measured in the 2nd trimester (58, 155). This may suggest that the association between sleep duration <7 hours and hypertensive disorders of pregnancy may be dependent upon the specific time of sleep measurements. With the inconsistency in these findings among the scant literature, there is uncertainty in whether sleep duration either in preconception or in mid-pregnancy is associated with risk of hypertensive disorders in pregnancy. Further work is needed to evaluate sleep duration from the preconception period through pregnancy using objective and longitudinal measures to better understand the potential time-varying associations between sleep duration and risk of hypertensive disorders of pregnancy.

Preconception Sleep Timing (Sleep Midpoint, Social Jet Lag), Shift Work (Night and Rotating) and Adverse Pregnancy Outcomes

In examining measures of sleep timing and circadian disruption, we first report on an association between preconception, early sleep midpoints (tertile 1 vs. tertile 2) and lower risk of the composite outcome. This association persisted among each of the individual adverse pregnancy outcomes, though the associations were imprecise. To our knowledge, no studies have evaluated the association between preconception sleep midpoints and risk of these adverse pregnancy outcomes. However, our findings

generally agree with results published from the large, NuMoM2b (n=10038) cohort. This study reported that later sleep midpoints in mid-pregnancy were associated with adverse pregnancy outcomes though associations were only suggestive (OR: 1.15, 0.92, 1.43) for hypertensive disorders of pregnancy (58, 155, 178). One potential explanation for the minor differences in our findings is that the NuMoM2b study measured sleep midpoints mid-pregnancy whereas sleep midpoints in EAGeR were measured in preconception, and it is possible the association between sleep midpoints and hypertensive disorders of pregnancy may be dependent upon when sleep midpoints were measured within gestation. Together, our results suggest that early sleep midpoints are associated with lower risk of adverse pregnancy outcomes whereas later sleep midpoints may be associated with greater risk. These findings and may preliminarily suggest that early sleep midpoints as early as the *preconception period* are associated as well. However, with some suggestive evidence for hypertensive disorders of pregnancy, there is a need for additional studies to evaluate sleep midpoints in association with hypertensive disorders of pregnancy. Future studies should incorporate validated questionnaires of chronotype as well as actigraphy to measure sleep midpoints throughout periconception to evaluate the acute and cumulative average effects of chronotype and sleep midpoints on hypertensive disorders of pregnancy.

In expanding on measures of sleep timing and circadian rhythms, we also report that neither preconception rotating nor night shift work was associated with risk of adverse pregnancy outcomes. In considering other adverse pregnancy outcomes, many studies note that shift work during pregnancy is associated with risk of preterm birth, and hypertensive disorders of pregnancy (179-184). Shift work in EAGeR was reported for the most recent and not necessarily the current job. In addition, we did not have information on work schedule patterns or shift work during pregnancy and it is possible women may have changed jobs and are no longer in shift work (185-187). In totality, shift work mid-pregnancy may be associated with adverse pregnancy outcomes. However, further studies of preconception shift work are needed that capture more detail about shift work (frequency of night schedules and rotation of night work) as well to understand if consistency of shift work in preconception could influence adverse pregnancy outcomes given the biological plausibility of circadian disruption from shift work influencing pregnancy in a critical window.

In order to capture other forms of mild circadian disruption, we also evaluated social jet lag during the preconception period. Among specific adverse pregnancy outcomes, social jet lag was associated with hypertensive disorders of pregnancy and GDM. To date, no studies have reported on associations between social jet lag and risk of adverse pregnancy outcomes, though other literature suggests social jet lag is associated with metabolic syndrome, obesity, and type 2 diabetes (59, 188-190). Given that social jet lag is related to cardiometabolic disorders, preconception social jet lag may be related to similar disorders in pregnancy but further study is needed to verify our findings given the lack of comparable studies.

Together, this evidence weakly supports a role of both sleep midpoint and circadian disruption during the preconception period with adverse pregnancy outcomes. Our work expands upon studies of mid-pregnancy sleep midpoint and adverse pregnancy outcomes to suggest that early sleep midpoints in the preconception period may be related to reduced risk of adverse pregnancy outcomes in mid-pregnancy. However, additional work is needed to investigate the role of both preconception sleep midpoints and social jet lag in adverse pregnancy outcomes throughout the periconceptional period to understand how circadian rhythms may longitudinally impact pregnancy.

Sleep may be related to many reproductive and pregnancy outcomes through the HPO axis (24, 92). Sleep behaviors influence the physiological processes of sleep, which include sleep homeostasis (sleep variability) and circadian rhythms, which control the timing of processes throughout the body (15-17, 21). These two processes interact to control the sleep-wake cycle and may influence the HPO axis (22, 23, 25, 26, 28-31). Poor sleep may influence reproductive hormone signaling, and thus many downstream reproductive functions such as ovulation, uterine growth, and implantation (21, 22, 31, 38, 191-194). We hypothesize that preconception usual sleep, which influences these reproductive processes, will be relevant near the time of conception, implantation, and early placental formation or may serve as a proxy of sleep at this critical and sensitive timepoint when morphological changes occur in the embryo and endometrium (130-132).

While we did not observe an association between preconception sleep behaviors and risk of pregnancy loss, there is biological plausibility for an association. One potential explanation is that many early pregnancy losses are due to genetic anomalies in embryos (172). These genetic anomalies may occur

due to random events unrelated to preconception sleep. Further, perhaps mild circadian disruptions in sleep may not be strong enough to influence pregnancy loss outcomes.

Our findings support the hypothesis that sleep behaviors during the preconception period may be weakly related to dysfunction in placental development and risk of adverse pregnancy outcomes. Poor sleep could alter maternal blood pressure leading to dysfunction in the placenta, or vice versa that the sleep could alter placental morphology in an early critical window leading to hypertensive states later in pregnancy (157, 195). Proper growth and development of the placenta is key to healthy gestation, and dysfunction in placental formation, which begins near weeks 3-5 of gestation, could lead to greater risk of adverse pregnancy outcomes (196-199). The influence of poor sleep in the preconception period on melatonin and clock genes in the early stages of placentation may provide evidence for a link between adverse pregnancy outcomes and sleep as early as the *preconception* period. The biological plausibility for this association may lie within circadian disruption due to social jet lag and potentially circadian disruption among women with later sleep midpoints (155). Circadian disruption (particularly among night shift workers) has been associated with lower melatonin and increased insulin resistance among non-diabetic women and may operate similarly as a mechanism for risk of GDM (200). Melatonin is essential for reducing inflammatory processes and oxidative processes throughout this early, sensitive window (22, 23, 25, 26, 201, 202). Cytotrophoblasts (cells that invade maternal uterine spiral arteries) and syncytiotrophoblasts (early, differentiated placental tissues), have receptors for melatonin (202). It is hypothesized that melatonin promotes growth and survivability of these cells as they develop into placental tissues and invade spiral arteries later in pregnancy (203). Clock genes also play a key role in this process (21, 112). Particularly, Rev-erbα (a clock gene specific clock protein produced from the BMAL1/CLOCK heterodimer) is related to placental decidualization in and plays a key role in placental growth and differentiation (204, 205). Circadian disruption may interfere with expression of these clock genes in critical processes of placental decidualization (206). In considering this biological plausibility, our work may preliminarily support the hypothesis that sleep during preconception could influence placental morphology and risk of adverse pregnancy outcomes in a sensitive window in the first weeks of pregnancy.

We did not observe an association between preconception measures of sleep and probability of live birth, despite hypothesized biological mechanisms linking preconception sleep to many events

throughout the reproductive process (24, 125). Previous evidence has shown a link between shorter sleep duration, and shift work with lower fecundability among pregnancy planning populations as well as a potential role of shorter and longer sleep duration with a lower probability of pregnancy among women seeking IVF treatment (94-100, 105). While sleep may influence the number of cycles it takes to become pregnant, it may not influence the probability of pregnancy. Once pregnancy is established, preconception sleep may not play a strong role in pregnancy survival and maintenance given random genetic anomalies account for many pregnancy losses (172). Indeed, our results preliminarily suggest that preconception sleep is not associated with early pregnancy losses. It is possible that mid-pregnancy sleep may play more of an important role in pregnancy maintenance and risk of stillbirth, but we are unable to consider this outcome in EAGeR due to the rarity of stillbirth. Furthermore, sleep was assessed during the preconception period to enable evaluation of questions related to fertility (i.e., probability of live birth) which requires following women attempting pregnancy and observing both who is and who is not successful. While assessments of mid-pregnancy sleep would be more proximal to the delivery of a live born infant, these assessments would only address questions regarding sleep on stillbirth or live birth, conditional on achieving pregnancy and having the pregnancy last through mid-pregnancy. Evaluating preconception sleep allows us to address pressing questions regarding fertility for pregnancy planning couples. Thus, given the biological plausibility of sleep in association with live birth, it will be important for future studies to address this important fertility endpoint specifically looking at the role of preconception sleep to verify our findings.

Strengths and Limitations

Our study has many strengths. Few studies have evaluated the role of preconception sleep in live birth, risk of pregnancy loss, and adverse pregnancy outcomes (135, 173). Our study importantly builds off work evaluating mid-pregnancy sleep and risk of adverse pregnancy outcomes and from mechanistic studies of sleep in pregnancy. In addition, the study design of EAGeR allows for high sensitivity detection of pregnancy losses through high sensitivity β-hCG testing to capture both recognized and unrecognized pregnancies and losses, offering a novel evaluation of preconception sleep with early pregnancy losses. Follow-up throughout EAGeR was rigorous resulting in minimal participant dropout. In addition, we used inverse probability weights to address potential selection biases from participant early withdrawal and for conditioning on pregnancy status (161, 166, 168, 169).

Our study also has a few limitations. Self-reported sleep may have been reported with error, but we expect this to be non-differentially misclassified with respect to live birth, pregnancy loss, and adverse pregnancy outcomes given sleep was measured prior to these endpoints. We only assessed baseline, usual sleep characteristics and cannot examine acute sleep mid-pregnancy or day-to-day variation in sleep. However, our study is among one of the few to assess preconception sleep characteristics in relation to pregnancy loss, live birth, and adverse pregnancy outcomes. Pregnancy is known to impact sleep and it is plausible that sleep during the preconception period may impact later outcomes and sleep during this critical window in pregnancy establishment and development is important to evaluate (207-211). Our interpretation of sleep is complicated by this time-varying nature of sleep that may have acute relationships with pregnancy outcomes that are stronger than those that occur in temporally distant etiological windows. Importantly, studies of sleep over pregnancy have noted that sleep characteristics measured by polysomnography and actigraphy do not vary considerably over gestation (209-212). On average sleep duration increases by only 30 minutes over the first trimester and sleep midpoints advance (become earlier) on average by 16-24 minutes over the first and second trimester, but return to pre-pregnancy levels by the third trimester (209, 210). However, women have also reported greater sleep disturbances and difficulty sleeping as early as the late first trimester (209, 211). Our measures of sleep midpoint and social jet lag were based on calculations using the sleep midpoints of weekend and weekday sleep and assuming that weekend sleep midpoints represent the midpoints of days "free" from work or the influence of an alarm clock (4, 6). Unfortunately, we did not have information on work or school schedules to determine whether weekends were in fact "free days." Our measures of shift work only queried most recent rather than current shift work and did not capture shift work schedules. It is possible our shift work measure may not adequately characterize circadian disruption due to shift work and may explain why we did not observe associations for shift work, but mild circadian disruption from social jet lag was associated with some outcomes. Our analyses of pregnancy loss and adverse pregnancy outcomes had limited power due to the few participants experiencing a pregnancy loss, and of those who did not have a pregnancy loss, few women developed hypertensive disorders of pregnancy, preterm birth, or GDM. For preterm birth in particular, we did not have enough power to examine spontaneous or medically indicated preterm births in stratified analyses. To address this limitation, we combined these outcomes into a composite outcome

representing the risk for any of these adverse pregnancy outcomes. This is in line with our hypothesis that many adverse pregnancy outcomes are of similar placental origin and helped to improve precision (125, 133, 134). Lastly, the EAGeR population was comprised of mostly healthy, white women attempting pregnancy who had a history of pregnancy loss, which may limit our generalizability. However, almost 30% of reproductive age women have experienced a prior pregnancy loss and thus represent a large proportion of women to whom these results would be generalizable (83). Future research among diverse populations is warranted.

Conclusion

Our results preliminarily suggest that sleep duration, sleep midpoint, social jet lag, and shift work during the preconception period may not be associated with the probability of delivering a live born infant or risk of pregnancy loss. Importantly our work extends upon prior studies of mid-pregnancy sleep and pregnancy loss through characterizing early and unrecognized pregnancy losses, which comprise almost 80% of pregnancy losses. In addition, our work weakly suggests that preconception sleep midpoint and social jet lag may be associated with adverse pregnancy outcomes, specifically preterm birth, and hypertensive disorders of pregnancy, though based on very limited case numbers. These outcomes may be affected by physiological processes that crossover from preconception into early pregnancy establishment and may plausibly be impacted by sleep behaviors in the preconception period. Future research should prospectively follow a large cohort of pregnancy-planning women using objective, longitudinal sleep measures to clarify the usual, acute, and cumulative average effects of sleep behaviors on pregnancy loss, adverse pregnancy outcomes, and live birth throughout the periconceptional period.

Tables

Chapter 1 Tables

Table 1.1. List of sleep variables, assessment method, and operationalization in EAGeR			
Variable	Assessment Method	Calculation	Operationalization
Sleep Duration	Baseline Usual Sleep Questionnaire	SleepDuration=RiseTime-BedTime-TimetoFallAsleep; Duration=[(5 x $duration_{weekday}$)+(2 x $duration_{weekend}$)]/7	Categorical (5 levels): <6, 6-7, 7-8, 8-9, and ≥ 9 hours
Shift Work Measures			
Night Shift Work	At your most recent job, have you had night work "defined as work in which most of the hours (>50%) are in the evening (between 4 p.m. and midnight) or at night (between midnight and 8 a.m.)"	N/A	Binary: Night Shift Work, Not Night Shift Work
Rotating Shift Work	At your most recent job, have you had rotating shift work "defined as work schedules in which the time changes between days, evenings and/or nights"	N/A	Binary: Rotating Shift Work, Not Rotating Shift Work
Sleep Timing Measures			
Sleep Midpoint	Baseline Usual Sleep Questionnaire	Midpoint=$midpoint_{weekend}$ - 0.5 x ($duration_{weekend}$ - Duration) If $midpoint_{weekend} \leq midpoint_{week}$ then Midpoint=$midpoint_{weekend}$	Tertiles
Social Jet Lag	Baseline Usual Sleep Questionnaire	Social Jet Lag = |$midpoint_{weekend}$ - $midpoint_{weekday}$|	Categorical (3 levels): <1, 1-2, and ≥ 2 hours

Table 1.2. Association between sleep characteristics and risk of anovulation; EAGeR Study (2006-2012) (n=1200)[ab]

	Anovulation n=496 of 3784	Unadjusted			Multivariate adjusted[c]			Restricted to 1st 2 cycles[c]		
Sleep Characteristics	n (%)	RR	95% CI		RR	95% CI		RR	95% CI	
Sleep Duration										
Sleep <6 hours (n=129)	16 (12.4%)	0.97	0.56	1.68	0.80	0.45	1.41	0.97	0.53	1.79
Sleep 6-7 hours (n=403)	64 (15.9%)	1.28	0.93	1.78	1.19	0.87	1.62	1.24	0.87	1.76
Sleep 7-8 hours (n=1491)	191 (12.8%)	Ref	--	--	Ref	--	--	Ref	--	--
Sleep 8-9 hours (n=1375)	180 (13.1%)	1.02	0.82	1.28	1.04	0.83	1.29	1.04	0.80	1.34
Sleep ≥9 hours (n=369)	42 (11.4%)	1.04	0.73	1.50	1.03	0.71	1.51	0.97	0.64	1.47
Sleep Midpoint										
Sleep Midpoint Tertile 1 (n=1290)	140 (10.9%)	0.90	0.70	1.16	0.90	0.70	1.15	0.86	0.64	1.15
Sleep Midpoint Tertile 2 (n=1230)	148 (12.0%)	Ref	--	--	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=1247)	205 (16.4%)	1.39	1.10	1.76	1.29	1.02	1.63	1.19	0.92	1.55
Social Jet Lag (hours)										
Social Jet Lag <1 hour (n=2387)	303 (12.7%)	Ref	--	--	Ref	--	--	Ref	--	--
Social Jet Lag 1-2 hour (n=1021)	139 (13.6%)	1.24	0.99	1.55	1.12	0.89	1.42	1.09	0.84	1.41
Social Jet Lag ≥2 hour (n=359)	51 (14.2%)	1.18	0.86	1.62	1.01	0.73	1.41	0.84	0.56	1.26
Rotating Shift Work										
Not Rotating Shift Work (n=3085)	398 (12.9%)	Ref	--	--	Ref	--	--	Ref	--	--
Rotating Shift Work (n=614)	92 (15.0%)	1.13	0.89	1.43	1.15	0.90	1.47	1.40	1.07	1.84
Night Shift Work										
Not Night Shift Work (n=2871)	362 (12.6%)	Ref	--	--	Ref	--	--	Ref	--	--
Night Shift Work (n=826)	128 (15.5%)	1.23	0.99	1.53	1.20	0.96	1.50	1.38	1.07	1.76

[a]Imputed data for missing sleep and covariates were used in this analysis
[b]Models are weighted for the inverse of the number of contributed cycles
[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), exercise (categorical; Low, Mid, High), alcohol (binary; Yes, No), caffeine (Categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No)

Table 1.3. Association between sleep duration and reproductive hormones using GEE with repeated measures across the menstrual cycle[ab]

		Sleep ≤6 hours		Sleep 6-7 hours		Sleep 7-8 hours	Sleep 8-9 hours		Sleep ≥ 9 hours	
	Model	PD	95% CI	PD	95% CI	Ref	PD	95% CI	PD	95% CI
FSH	Unadjusted	37	(11, 69)	6	(-7, 20)	Ref	9	(0, 19)	22	(6, 39)
	Adjusted[c]	35	(10, 66)	6	(-7, 20)	Ref	8	(-1, 17)	11	(-4, 27)
Ovulatory LH	Unadjusted	32	(-18, 113)	-14	(-36, 14)	Ref	20	(-2, 46)	15	(-13, 51)
	Adjusted[c]	63	(2, 161)	-6	(-29, 24)	Ref	16	(-4, 41)	3	(-23, 37)
Luteal PDG	Unadjusted	-4	(-38, 47)	-5	(-23, 16)	Ref	-6	(-17, 8)	-9	(-24, 9)
	Adjusted[c]	-3	(-36, 46)	-2	(-19, 19)	Ref	-6	(-17, 8)	-15	(-29, 3)
E2	Unadjusted	7	(-7, 24)	0	(-9, 11)	Ref	2	(-4, 8)	0	(-8, 9)
	Adjusted[c]	4	(-10, 21)	1	(-8, 11)	Ref	3	(-3, 9)	0	(-8, 9)
E1G	Unadjusted	6	(-14, 31)	-2	(-15, 13)	Ref	0	(-8, 10)	-1	(-13, 12)
	Adjusted[c]	4	(-16, 29)	-2	(-15, 14)	Ref	0	(-8,9)	-6	(-18, 7)

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]PD and 95% CI weighted for the number of contributed cycles

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol use (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), seep aid use (binary; Yes, No), exercise (categorical; Low, Moderate, High), and t, t^2, t^3, t^4, where t=day/cycle_length (polynomial terms of t are used to mimic hormone curvature)

Table 1.4. Association between rotating and night shift work and reproductive hormones using GEE with repeated measures across the menstrual cycle[ab]

	Model	Rotating Shift Work vs. Not Rotating Shift Work		Night Shift Work vs. Not Night Shift Work	
		PD	95% CI	PD	95% CI
FSH	Unadjusted	1	(-9, 13)	3	(-6, 13)
	Adjusted[c]	2	(-8, 13)	1	(-8, 11)
Ovulatory LH	Unadjusted	-21	(-38, 0)	-6	(-23, 14)
	Adjusted[c]	-21	(-37, 0)	-8	(-24, 13)
Luteal PDG	Unadjusted	-10	(-23, 5)	-14	(-25, -1)
	Adjusted[c]	-14	(-26, 1)	-19	(-29, -7)
E2	Unadjusted	1	(-7, 8)	-3	(-9, 3)
	Adjusted[c]	1	(-6, 8)	-5	(-10, 2)
E1G	Unadjusted	0	(-10, 10)	-9	(-17, 0)
	Adjusted[c]	-1	(-11, 9)	-13	(-21, -4)

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]PD and 95% CI weighted for the number of contributed cycles

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), exercise (categorical; Low, Moderate, High), and t, t^2, t^3, t^4, where t=day/cycle_length (polynomial terms of t are used to mimic hormone curvature)

Table 1.5. Association between sleep midpoint and reproductive hormones using GEE with repeated measures across the menstrual cycle[ab]

	Model	Midpoint Tertile 1		Midpoint Tertile 2	Midpoint Tertile 3	
		PD	95% CI	Ref	PD	95% CI
FSH	Unadjusted	5	(-4, 15)	Ref	8	(-2, 18)
	Adjusted[c]	7	(-2, 16)	Ref	6	(-3, 16)
Ovulatory LH	Unadjusted	18	(-5, 45)	Ref	0	(-19, 23)
	Adjusted[c]	17	(-5, 43)	Ref	-2	(-21, 21)
Luteal PDG	Unadjusted	6	(-8, 22)	Ref	-8	(-20, 7)
	Adjusted[c]	5	(-8, 21)	Ref	-4	(-17, 11)
E2	Unadjusted	10	(3, 17)	Ref	-2	(-8, 5)
	Adjusted[c]	10	(3, 17)	Ref	-1	(-8, 5)
E1G	Unadjusted	4	(-6, 14)	Ref	2	(-7, 12)
	Adjusted[c]	5	(-5, 15)	Ref	1	(-8, 11)

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]PD and 95% CI weighted for number of contributed cycles

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), exercise (categorical; Low, Moderate, High), and t, t^2, t^3, t^4, where t=day/cycle_length (polynomial terms of t are used to mimic hormone curvature)

Table 1.6. Association between social jet lag and reproductive hormones using GEE with repeated measures across the menstrual cycle[ab]

	Model	Social Jet Lag 0-1 hour	Social Jet Lag 1-2 hours		Social Jet Lag ≥2 hours	
			PD	95% CI	PD	95% CI
FSH	Unadjusted	Ref	0	(-9, 9)	-8	(-20, 6)
	Adjusted[c]	Ref	1	(-8, 10)	-11	(-23, 1)
Ovulatory LH	Unadjusted	Ref	6	(-13, 28)	-6	(-30, 26)
	Adjusted[c]	Ref	5	(-14, 28)	-9	(-32, 22)
Luteal PDG	Unadjusted	Ref	-10	(-22, 3)	2	(-17, 25)
	Adjusted[c]	Ref	-2	(-15, 13)	10	(-11, 37)
E2	Unadjusted	Ref	3	(-3, 9)	-1	(-9, 8)
	Adjusted[c]	Ref	2	(-4, 9)	-2	(-11, 7)
E1G	Unadjusted	Ref	4	(-5, 13)	6	(-7, 20)
	Adjusted[c]	Ref	3	(-6, 12)	4	(-8, 19)

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]PD and 95% CI weighted for number of contributed cycles

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (Categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (Categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), exercise (Categorical; Low, Moderate, High), and t, t^2, t^3, t^4, where t=day/cycle_length (polynomial terms of t are used to mimic hormone curvature)

Chapter 1 Supplementary Tables

Table 1.1S. Characteristics of participants (N=1192) by baseline sleep duration; EAGeR Study (2006-2012)

Characteristics	Sleep <6 hours n=45	Sleep 6-7 hours n=131	Sleep 7-8 hours n=465	Sleep 8-9 hours n=420	Sleep ≥9 hours n=131
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	29.8 (5.4)	28.7 (5.3)	29.5 (4.6)	28.4 (4.6)	26.6 (4.5)
BMI (kg/m^2)	29.7 (8.1)	27.0 (6.9)	26.4 (6.1)	26.1 (6.6)	25.0 (6.2)
Sleep Midpoint (Hour:Min AM)**	4:01AM (3:10AM, 5:15AM)	3:36AM (2:59AM, 4:26AM)	3:33AM (2:58AM, 4:05AM)	3:34 AM (2:54AM, 4:23AM)	4:10AM (3:22 AM, 5:15AM)
Social Jet Lag (minutes)**	30 (0, 90)	60 (15, 113)	60 (15, 90)	45 (0, 90)	45 (0, 83)
	n (%)	n (%)	n (%)	n (%)	n (%)
Sleep Midpoint					
Tertile 1	12 (26.7)	40 (30.5)	181 (38.9)	139 (33.1)	22 (16.8)
Tertile 2	13 (28.9)	46 (35.1)	159 (34.2)	141 (33.6)	40 (30.5)
Tertile 3	20 (44.4)	45 (34.4)	125 (26.9)	140 (33.3)	69 (52.7)
Parity					
Nulliparous	16 (35.6)	58 (44.3)	199 (42.8)	180 (42.9)	56 (42.8)
1	14 (31.1)	47 (35.9)	155 (33.3)	157 (37.4)	47 (35.9)
2+	15 (33.3)	26 (19.9)	111 (23.9)	83 (19.8)	28 (21.4)
Marijuana Use	3 (6.7)	5 (3.8)	20 (4.3)	17 (4.1)	13 (9.9)
Opioid Use	9 (20.0)	9 (6.9)	32 (7.0)	22 (5.3)	13 (10.3)
Antidepressant Use	5 (11.1)	15 (11.5)	72 (15.5)	79 (18.8)	32 (24.4)
Sleep Aid Use	2 (4.4)	4 (3.1)	17 (3.7)	18 (4.3)	12 (9.5)
More than high school education	34 (75.6)	110 (84.0)	420 (90.3)	369 (87.9)	97 (74.6)
Race					
White	41 (91.1)	122 (93.1)	444 (95.5)	403 (96.0)	120 (91.6)
Cotinine (Smoking)	13 (28.9)	21 (16.0)	44 (9.6)	39 (9.4)	19 (15.1)
Alcohol consumption in past year					
Never	24 (53.3)	90 (69.8)	305 (66.2)	290 (69.2)	88 (67.7)
Sometimes	21 (46.7)	37 (28.7)	148 (32.1)	121 (28.9)	39 (30.0)
Often	0 (0.0)	2 (1.6)	8 (1.7)	8 (1.9)	3 (2.3)
Currently Employed	31 (75.6)	107 (84.9)	359 (78.6)	302 (74.4)	74 (60.7)
Caffeine Consumption					
Nondrinker	4 (8.9)	30 (22.9)	120 (25.8)	114 (27.1)	31 (23.7)
1-3 Cups/day	26 (57.8)	77 (58.8)	269 (57.9)	249 (59.3)	72 (55.0)
≥ 3 Cups/day	15 (33.3)	24 (18.3)	76 (16.3)	57 (13.6)	28 (21.4)
Stress					
Quartile 1	8 (22.9)	19 (17.3)	96 (24.2)	94 (26.2)	30 (30.0)
Quartile 2	9 (25.7)	24 (21.8)	93 (23.4)	100 (27.9)	23 (23.0)

Quartile 3	9 (25.7)	31 (28.2)	113 (28.5)	83 (23.1)	19 (19.0)
Quartile 4	9 (25.7)	36 (32.7)	95 (23.9)	82 (22.8)	28 (28.0)
Exercise					
Low	7 (15.6)	27 (20.6)	118 (25.4)	110 (26.2)	47 (35.9)
Moderate	15 (33.3)	52 (39.7)	202 (43.4)	172 (41.0)	42 (32.1)
High	23 (51.1)	52 (39.7)	145 (31.2)	138 (32.9)	42 (32.1)
Rotating Shift Work	15 (36.6)	19 (15.2)	68 (14.9)	67 (16.5)	32 (26.5)
Night Shift Work	17 (42.5)	31 (24.8)	97 (21.3)	83 (20.5)	43 (35.3)
Season					
Winter	7 (15.6)	29 (22.1)	101 (21.7)	99 (23.6)	39 (29.8)
Spring	12 (26.7)	33 (25.2)	150 (32.3)	114 (27.1)	33 (25.2)
Summer	13 (28.9)	33 (25.2)	95 (20.4)	94 (22.4)	34 (26.0)
Fall	13 (28.9)	36 (27.5)	119 (25.6)	113 (26.9)	25 (19.1)

** Median (Q1, Q3)

Table 1.2S. Characteristics of participants (N=1192) by tertile of sleep midpoint; EAGeR Study (2006-2012)

Characteristics	Sleep Midpoint Tertile 1 n=394	Sleep Midpoint Tertile 2 n=399	Sleep Midpoint Tertile 3 n=399
	Mean (±SD)	Mean (±SD)	Mean (±SD)
Age (years)	30.0 (4.7)	29.0 (4.6)	27.2 (4.5)
BMI (kg/m^2)	26.5 (6.6)	26.1 (6.5)	26.3 (6.5)
Social Jet Lag (Minutes)**	30 (0, 60)	45 (0, 90)	68 (30, 113)
Sleep Midpoint (Hour:Min AM)**	2:45 AM (2:22 AM, 3:00 AM)	3:36 AM (3:25 AM, 3:48AM)	4:40 AM (4:15 AM, 5:16 AM)
Sleep Duration (Hours)**	7.90 (7.40, 8.40)	7.85 (7.32, 8.45)	8.00 (7.42, 8.72)
	n (%)	n (%)	n (%)
Sleep Duration			
<6 hours	12 (3.1)	13 (3.3)	20 (5.0)
6-7 hours	40 (10.2)	46 (11.5)	45 (11.3)
7-8 hours	181 (45.9)	159 (39.9)	125 (31.3)
8-9 hours	139 (35.3)	141 (35.3)	140 (35.1)
≥9 hours	22 (5.6)	40 (10.0)	69 (17.3)
Parity			
Nulliparous	150 (38.1)	160 (40.1)	199 (49.9)
1	145 (36.8)	147 (36.8)	128 (32.1)
2+	99 (25.1)	92 (23.1)	72 (18.1)
Marijuana Use	9 (2.3)	18 (4.5)	31 (7.8)
Opioid Use	19 (4.9)	26 (6.7)	40 (10.2)
Antidepressant Use	58 (14.7)	72 (18.1)	73 (18.3)
Sleep Aid Use	14 (3.6)	15 (3.8)	24 (6.1)
More than high school education	349 (88.6)	354 (88.7)	327 (82.2)
Race			
White	378 (95.9)	378 (94.7)	374 (93.7)
Cotinine (Smoking)	38 (9.7)	32 (8.2)	66 (16.8)
Alcohol consumption in past year			
Never	261 (66.8)	270 (68.2)	266 (67.0)
Sometimes	121 (31.0)	123 (31.1)	122 (30.7)
Often	9 (2.3)	3 (0.8)	9 (2.3)
Currently Employed	306 (79.5)	283 (73.7)	284 (74.2)
Caffeine Consumption			
Nondrinker	111 (28.2)	106 (26.6)	82 (20.6)
1-3 Cups/day	224 (56.9)	233 (58.4)	236 (59.2)
≥ 3 Cups/day	59 (15.0)	60 (15.0)	81 (20.3)

Stress			
Quartile 1	87 (24.4)	94 (27.7)	75 (22.5)
Quartile 2	93 (26.1)	77 (22.7)	88 (26.4)
Quartile 3	93 (26.1)	96 (28.3)	70 (21.0)
Quartile 4	83 (23.3)	72 (21.2)	101 (30.2)
Exercise			
Low	100 (25.4)	110 (27.6)	99 (24.8)
Moderate	165 (41.9)	159 (39.9)	159 (39.9)
High	129 (32.7)	130 (32.6)	141 (35.3)
Rotating Shift Work	55 (14.3)	66 (17.3)	80 (21.0)
Night Shift Work	71 (18.5)	86 (22.6)	114 (29.8)
Season			
Winter	93 (23.6)	101 (25.3)	81 (20.3)
Spring	116 (29.4)	103 (25.8)	123 (30.8)
Summer	80 (20.3)	86 (21.6)	103 (25.8)
Fall	105 (26.6)	109 (27.3)	92 (23.1)

** Median (Q1, Q3)

Chapter 2 Tables

Table 2.1. List of sleep variables, assessment method, and operationalization in EAGeR			
Variable	Assessment Method	Calculation	Operationalization
Sleep Duration	Baseline Usual Sleep Questionnaire	SleepDuration=RiseTime-BedTime-TimetoFallAsleep; Duration=[(5 x $duration_{weekday}$)+(2 x $duration_{weekend}$)]/7	Categorical (5 levels): <6, 6-<7, 7-8, 8-9, and ≥9 hours, Continuous
Shift Work Measures			
Night Shift Work	At your most recent job, have you had night work "defined as work in which most of the hours (>50%) are in the evening (between 4 p.m. and midnight) or at night (between midnight and 8 a.m.)"	N/A	Binary: Night Shift Work, Not Night Shift Work
Rotating Shift Work	At your most recent job, have you had rotating shift work "defined as work schedules in which the time changes between days, evenings and/or nights"	N/A	Binary: Rotating Shift Work, Not Rotating Shift Work
Sleep Timing Measures			
Sleep Midpoint	Baseline Usual Sleep Questionnaire	Midpoint=$midpoint_{weekend}$ - 0.5 x ($duration_{weekend}$ - Duration) If $midpoint_{weekend} \leq midpoint_{week}$ then Midpoint=$midpoint_{weekend}$	Tertiles, Continuous
Social Jet Lag	Baseline Usual Sleep Questionnaire	Social Jet Lag = |$midpoint_{weekend}$ - $midpoint_{weekday}$|	Categorical (3 levels): <1, 1-2, and ≥ 2 hours, Continuous

Table 2.2. Characteristics of participants (N=1220) by baseline sleep duration; EAGeR Study (2006-2012)

Characteristics	Sleep <6 hours n=46	Sleep 6-7 hours n=134	Sleep 7-8 hours n=473	Sleep 8-9 hours n=429	Sleep ≥9 hours n=138
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	29.9 (5.4)	28.6 (5.3)	29.6 (4.6)	28.6 (4.6)	26.5 (4.5)
BMI (kg/m^2)	29.4 (8.2)	27.2 (6.9)	26.5 (6.1)	26.0 (6.6)	25.1 (6.2)
Social Jet Lag (Hours:Min)	1:09 (2:34)	1:14 (1:14)	0:58 (0:58)	0:56 (1:12)	1:10 (1:42)
Cycles until 1st hCG Pregnancy**	2 (1, 3)	2 (1, 4)	2 (1, 4)	2 (1, 3)	3 (1,3)
	n (%)	n (%)	n (%)	n (%)	n (%)
Sleep Midpoint					
Tertile 1	12 (26.1)	40 (29.9)	184 (38.9)	148 (34.5)	22 (15.9)
Tertile 2	13 (28.3)	46 (34.3)	161 (34.0)	141 (32.9)	42 (30.4)
Tertile 3	21 (45.7)	48 (35.8)	128 (27.1)	140 (32.6)	74 (53.6)
Achieved Pregnancy	26 (56.5)	88 (65.7)	319 (67.4)	279 (65.0)	83 (60.1)
Parity					
Nulliparous	16 (34.8)	59 (44.0)	205 (43.3)	183 (42.7)	58 (42.0)
1	14 (30.4)	49 (36.6)	156 (33.0)	161 (37.5)	51 (37.0)
2+	16 (34.8)	26 (19.4)	112 (23.7)	85 (19.8)	29 (21.0)
Marijuana Use	3 (6.5)	5 (3.7)	20 (4.2)	17 (4.0)	14 (10.1)
Opioid Use	9 (19.6)	9 (6.7)	32 (6.9)	22 (5.2)	13 (9.8)
Antidepressant Use	5 (10.9)	15 (11.2)	73 (15.4)	80 (18.7)	33 (23.9)
Sleep Aid Use	2 (4.4)	4 (3.0)	17 (3.7)	18 (4.3)	12 (9.0)
More than high school education	35 (76.1)	111 (82.8)	428 (90.5)	378 (88.1)	100 (73.0)
Race					
White	42 (91.3)	125 (93.3)	452 (95.6)	412 (96.0)	126 (91.3)
Cotinine (Smoking)	13 (28.3)	23 (17.2)	43 (9.2)	40 (9.4)	22 (16.5)
Alcohol consumption in past year					
Never	24 (52.2)	92 (69.7)	304 (64.8)	291 (68.0)	95 (69.3)
Sometimes	22 (47.8)	38 (28.8)	156 (33.3)	125 (29.2)	39 (28.5)
Often	0 (0.0)	2 (1.5)	9 (1.9)	12 (2.8)	3 (2.2)
Currently Employed	31 (73.8)	109 (84.5)	366 (78.9)	307 (74.0)	79 (61.2)
Caffeine Consumption					
Nondrinker	4 (8.7)	30 (22.4)	122 (25.8)	116 (27.0)	33 (23.9)
1-3 Cups/day	27 (58.7)	80 (59.7)	273 (57.7)	256 (59.7)	77 (55.8)
≥ 3 Cups/day	15 (32.6)	24 (17.9)	78 (16.5)	57 (13.3)	28 (20.3)
Sexual Intercourse Frequency Past Month					
Weekly or more	30 (76.9)	97 (78.2)	320 (74.8)	295 (74.7)	104 (83.2)
Less than weekly	9 (23.1)	27 (21.8)	108 (25.2)	100 (25.3)	21 (16.8)

Stress					
Quartile 1	8 (22.2)	20 (17.7)	98 (24.2)	97 (26.4)	33 (30.8)
Quartile 2	10 (27.8)	26 (23.0)	95 (23.5)	102 (27.7)	25 (23.4)
Quartile 3	9 (25.0)	31 (27.4)	115 (28.4)	84 (22.8)	20 (18.7)
Quartile 4	9 (25.0)	36 (31.9)	97 (24.0)	85 (23.1)	29 (27.1)
Exercise					
Low	8 (17.4)	28 (20.9)	122 (25.8)	114 (26.6)	48 (34.8)
Moderate	15 (32.6)	53 (39.6)	206 (43.6)	176 (41.0)	46 (33.3)
High	23 (50.0)	53 (39.6)	145 (30.7)	139 (32.4)	44 (31.9)
Rotating Shift Work	16 (38.1)	19 (14.8)	69 (14.9)	67 (16.2)	36 (28.1)
Night Shift Work	17 (41.5)	31 (24.2)	97 (21.0)	83 (20.1)	46 (35.7)
Season					
Winter	7 (15.2)	29 (21.6)	102 (21.6)	98 (22.8)	41 (29.7)
Spring	12 (26.1)	34 (25.4)	152 (32.1)	115 (26.8)	35 (25.4)
Summer	14 (30.4)	34 (25.4)	99 (20.9)	96 (22.4)	34 (24.6)
Fall	13 (28.3)	37 (27.6)	120 (25.4)	120 (28.0)	28 (20.3)

** Median (Q1, Q3)

Table 2.3. Association between sleep characteristics and fecundability; EAGeR Study (2006-2012) (n=1228)[a]

	Achieved Pregnancy	Unadjusted			Multivariate adjusted[b]		
Sleep Characteristics	n (%)	FOR	95% CI		FOR	95% CI	
Sleep Duration							
Sleep <6 hours (n=46)	26 (56.5%)	0.99	0.63	1.54	1.06	0.67	1.66
Sleep 6-7 hours (n=134)	88 (65.7%)	1.09	0.83	1.42	1.13	0.86	1.48
Sleep 7-8 hours (n=476)	321 (67.4%)	Ref	--	--	Ref	--	--
Sleep 8-9 hours (n=430)	279 (64.9%)	0.99	0.82	1.18	0.94	0.78	1.13
Sleep ≥9 hours (n=134)	81 (60.5%)	0.83	0.63	1.10	0.80	0.60	1.07
Rotating Shift Work							
Not Rotating Shift Work (n=973)	640 (65.8%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=207)	144 (69.6%)	1.11	0.90	1.36	1.07	0.86	1.32
Night Shift Work							
Not Night Shift Work (n=903)	586 (64.9%)	Ref	--	--	Ref	--	--
Night Shift Work (n=275)	196 (71.3%)	1.20	0.99	1.45	1.17	0.96	1.42
Sleep Midpoint							
Sleep Midpoint Tertile 1 (n=406)	277 (68.2%)	1.01	0.83	1.22	1.00	0.82	1.21
Sleep Midpoint Tertile 2 (n=404)	272 (67.3%)	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=410)	246 (60.0%)	0.84	0.69	1.02	0.85	0.69	1.04
Sleep Midpoint Continuous (n=1220)	795 (65.2%)	0.95	0.90	1.01	0.96	0.90	1.02
Social Jet Lag							
Social Jet Lag <1 hour (n=777)	526 (67.7%)	Ref	--	--	Ref	--	--
Social Jet Lag 1-2 hours (n=321)	199 (62.0%)	0.83	0.69	0.99	0.92	0.75	1.12
Social Jet Lag ≥2 hours (n=122)	70 (57.4%)	0.81	0.61	1.07	0.91	0.68	1.22
Social Jet Lag Continuous (n=1220)	795 (65.2%)	0.90	0.83	0.97	0.92	0.86	1.00

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sexual intercourse frequency past month (binary; <weekly, weekly or more), sleep aid use (binary; Yes, No), and exercise (categorical; Low, Moderate, High)

Table 2.4. Association between sleep characteristics and fecundability in sensitivity analysis subcohorts; EAGeR Study (2006-2012)[ab]

	≤3 cycles (n=917)			Non-night or rotating shift workers (n=841)		
Sleep Characteristics	FOR	95% CI		FOR	95% CI	
Sleep Duration						
Sleep <6 hours	0.99	0.57	1.70	0.59	0.29	1.18
Sleep 6-7 hours	1.02	0.74	1.39	1.13	0.81	1.56
Sleep 7-8 hours	Ref	--	--	Ref	--	--
Sleep 8-9 hours	0.89	0.73	1.10	0.90	0.72	1.12
Sleep ≥9 hours	0.70	0.51	0.97	0.64	0.43	0.96
Rotating Shift Work						
No Rotating Shift Work	Ref	--	--			
Rotating Shift Work	1.07	0.84	1.38			
Night Shift Work						
No Night Shift Work	Ref	--	--			
Night Shift Work	1.13	0.91	1.41			
Sleep Midpoint						
Sleep Midpoint Tertile 1	0.99	0.80	1.23	0.86	0.68	1.09
Sleep Midpoint Tertile 2	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3	0.85	0.68	1.06	0.86	0.68	1.09
Sleep Midpoint Continuous	0.93	0.86	0.99	0.96	0.87	1.05
Social Jet Lag						
Social Jet Lag <1 hour	Ref	--	--	Ref	--	--
Social Jet Lag 1-2 hour	0.87	0.70	1.08	1.01	0.80	1.27
Social Jet Lag ≥2 hour	0.84	0.59	1.18	1.23	0.86	1.75
Social Jet Lag Continuous	0.90	0.82	0.98	0.98	0.88	1.10

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sexual intercourse frequency past month (binary; <weekly, weekly or more), sleep aid use (binary; Yes, No), and exercise (categorical; Low, Mid, High)

Chapter 2 Supplementary Tables

Table 2.1S. Characteristics of participants (N=1220) by tertile of sleep midpoint; EAGeR Study (2006-2012)

Characteristics	Sleep Midpoint Tertile 1 n=406	Sleep Midpoint Tertile 2 n=404	Sleep Midpoint Tertile 3 n=410
	Mean (±SD)	Mean (±SD)	Mean (±SD)
Age (years)	30.1 (4.8)	29.1 (4.6)	27.2 (4.6)
BMI (kg/m^2)	26.5 (6.5)	26.1 (6.5)	26.4 (6.5)
Social Jet Lag (Minutes)**	30 (0, 60)	45 (4, 90)	65 (30, 108)
Sleep Midpoint (Hour:Min AM)**	2:45 AM (2:20 AM, 3:00 AM)	3:36 AM (3:25 AM, 3:48AM)	4:40 AM (4:21 AM, 5:17 AM)
Sleep Duration (Hours)**	7.92 (7.42, 8.40)	7.87 (7.30, 8.45)	8.00 (7.42, 8.72)
Cycles Until 1st hCG Pregnancy**	2 (1, 4)	2 (1, 3)	2 (1, 4)
	n (%)	n (%)	n (%)
Sleep Duration			
<6 hours	12 (3.0)	13 (3.2)	21 (5.1)
6-7 hours	40 (9.9)	46 (11.4)	48 (11.7)
7-8 hours	186 (45.8)	161 (39.9)	129 (31.5)
8-9 hours	146 (36.0)	143 (35.4)	141 (34.4)
≥9 hours	22 (5.4)	41 (10.2)	71 (17.3)
Parity			
Nulliparous	155 (38.2)	162 (40.1)	204 (49.8)
1	150 (37.0)	148 (36.6)	133 (32.4)
2+	101 (24.9)	94 (23.3)	73 (17.8)
Marijuana Use	9 (2.2)	18 (4.5)	32 (7.8)
Opioid Use	19 (4.7)	26 (6.6)	40 (9.9)
Antidepressant Use	61 (15.0)	72 (17.8)	73 (17.8)
Sleep Aid Use	14 (3.5)	15 (3.8)	24 (5.9)
More than high school education	359 (88.4)	358 (88.6)	335 (81.9)
Race			
White	390 (96.1)	383 (94.8)	384 (93.7)
Cotinine (Smoking)	39 (9.7)	32 (8.1)	70 (17.3)
Alcohol consumption in past year			
Never	264 (65.5)	271 (67.6)	271 (66.4)
Sometimes	127 (31.5)	125 (31.2)	128 (31.4)
Often	12 (3.0)	5 (1.3)	9 (2.2)
Currently Employed	316 (79.6)	286 (73.7)	290 (73.6)

Caffeine Consumption			
Nondrinker	114 (28.1)	107 (26.5)	84 (20.5)
1-3 Cups/day	232 (57.1)	237 (58.7)	244 (59.5)
≥ 3 Cups/day	60 (14.8)	60 (14.9)	82 (20.0)
Stress			
Quartile 1	87 (24.4)	94 (27.7)	75 (22.5)
Quartile 2	93 (26.1)	77 (22.7)	88 (26.4)
Quartile 3	93 (26.1)	96 (28.3)	70 (21.0)
Quartile 4	83 (23.3)	72 (21.2)	101 (30.2)
Exercise			
Low	105 (25.9)	112 (27.7)	103 (25.1)
Moderate	170 (41.9)	162 (40.1)	164 (40.0)
High	131 (32.3)	130 (32.2)	143 (34.9)
Rotating Shift Work	57 (14.4)	66 (17.1)	84 (21.4)
Night Shift Work	72 (18.2)	86 (22.3)	116 (29.5)
Season			
Winter	94 (23.2)	101 (25.0)	82 (20.0)
Spring	119 (29.3)	104 (25.7)	125 (30.5)
Summer	83 (20.4)	88 (21.8)	106 (25.9)
Fall	110 (27.1)	111 (27.5)	97 (23.7)

** Median (Q1, Q3)

Chapter 3 Tables

Table 3.1. List of sleep variables, assessment method, and operationalization in EAGeR			
Variable	Assessment Method	Calculation	Operationalization
Sleep Duration	Baseline Usual Sleep Questionnaire	SleepDuration=RiseTime-BedTime-TimetoFallAsleep; Duration=[(5 x $duration_{weekday}$)+(2 x $duration_{weekend}$)]/7	Categorical (5 levels): <6, 6-7, 7-8, 8-9, and ≥9 hours (Live Birth), <7, 7-9, and ≥9 hours (Pregnancy Loss and Adverse Pregnancy Outcomes), Continuous
Shift Work Measures			
Night Shift Work	At your most recent job, have you had night work "defined as work in which most of the hours (>50%) are in the evening (between 4 p.m. and midnight) or at night (between midnight and 8 a.m.)"	N/A	Binary: Night Shift Work, Not Night Shift Work
Rotating Shift Work	At your most recent job, have you had rotating shift work "defined as work schedules in which the time changes between days, evenings and/or nights"	N/A	Binary: Rotating Shift Work, Not Rotating Shift Work
Sleep Timing Measures			
Sleep Midpoint	Baseline Usual Sleep Questionnaire	Midpoint=$midpoint_{weekend}$ - 0.5 x ($duration_{weekend}$ - Duration) If $midpoint_{weekend} \leq midpoint_{week}$ then Midpoint=$midpoint_{weekend}$	Tertiles, Continuous
Social Jet Lag	Baseline Usual Sleep Questionnaire	Social Jet Lag = $\lvert midpoint_{weekend} - midpoint_{weekday} \rvert$	Categorical: <1, 1-2, and ≥ 2 hours (Live Birth), <1, ≥ 1 hours (Pregnancy Loss and Adverse Pregnancy Outcomes), Continuous

Table 3.2. Characteristics of participants (N=1220) by baseline sleep duration; EAGeR Study (2006-2012)

Characteristics	Sleep <6 hours n=46	Sleep 6-7 hours n=134	Sleep 7-8 hours n=473	Sleep 8-9 hours n=429	Sleep ≥9 hours n=138
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	29.9 (5.4)	28.6 (5.3)	29.6 (4.6)	28.6 (4.6)	26.5 (4.5)
BMI (kg/m^2)	29.4 (8.2)	27.2 (6.9)	26.5 (6.1)	26.0 (6.6)	25.1 (6.2)
Social Jet Lag (Hours)	1.15 (2.57)	1.23 (1.23)	0.97 (0.97)	0.93 (1.20)	1.17 (1.70)
	n (%)	n (%)	n (%)	n (%)	n (%)
Sleep Midpoint					
Tertile 1	12 (26.1)	40 (29.9)	184 (38.9)	148 (34.5)	22 (15.9)
Tertile 2	13 (28.3)	46 (34.3)	161 (34.0)	141 (32.9)	42 (30.4)
Tertile 3	21 (45.7)	48 (35.8)	128 (27.1)	140 (32.6)	74 (53.6)
Number Previous Live Births					
0	19 (41.3)	61 (45.5)	219 (46.3)	198 (46.2)	69 (50)
1	14 (30.4)	48 (35.8)	160 (33.8)	168 (39.2)	51 (37.0)
2	13 (28.3)	25 (18.7)	94 (19.9)	63 (14.7)	18 (13.0)
Number of Previous Losses					
1	29 (63.0)	93 (69.4)	324 (68.5)	285 (66.4)	89 (64.5)
2	17 (37.0)	41 (30.6)	149 (31.5)	144 (33.6)	49 (35.5)
Withdrew	8 (17.4)	19 (14.2)	44 (9.3)	37 (8.6)	27 (19.6)
Treatment Group					
Placebo	22 (47.8)	65 (48.5)	245 (51.8)	213 (49.7)	66 (47.8)
Aspirin	24 (52.2)	69 (51.5)	228 (48.2)	216 (50.4)	72 (52.2)
Married	37 (80.4)	120 (89.6)	444 (93.9)	400 (93.2)	115 (83.3)
Parity					
Nulliparous	16 (34.8)	59 (44.0)	205 (43.3)	183 (42.7)	58 (42.0)
1	14 (30.4)	49 (36.6)	156 (33.0)	161 (37.5)	51 (37.0)
2+	16 (34.8)	26 (19.4)	112 (23.7)	85 (19.8)	29 (21.0)
Marijuana Use	3 (6.5)	5 (3.7)	20 (4.2)	17 (4.0)	14 (10.1)
Opioid Use	9 (19.6)	9 (6.7)	32 (6.9)	22 (5.2)	13 (9.8)
Antidepressant Use	5 (10.9)	15 (11.2)	73 (15.4)	80 (18.7)	33 (23.9)
Sleep Aid Use	2 (4.4)	4 (3.0)	17 (3.7)	18 (4.3)	12 (9.0)
More than high school education	35 (76.1)	111 (82.8)	428 (90.5)	378 (88.1)	100 (73.0)
Race					

White	42 (91.3)	125 (93.3)	452 (95.6)	412 (96.0)	126 (91.3)
Cotinine (Smoking)	13 (28.3)	23 (17.2)	43 (9.2)	40 (9.4)	22 (16.5)
Alcohol consumption in past year					
Never	24 (52.2)	92 (69.7)	304 (64.8)	291 (68.0)	95 (69.3)
Sometimes	22 (47.8)	38 (28.8)	156 (33.3)	125 (29.2)	39 (28.5)
Often	0 (0.0)	2 (1.5)	9 (1.9)	12 (2.8)	3 (2.2)
Currently Employed	31 (73.8)	109 (84.5)	366 (78.9)	307 (74.0)	79 (61.2)
Caffeine Consumption					
Nondrinker	4 (8.7)	30 (22.4)	122 (25.8)	116 (27.0)	33 (23.9)
1-3 Cups/day	27 (58.7)	80 (59.7)	273 (57.7)	256 (59.7)	77 (55.8)
≥ 3 Cups/day	15 (32.6)	24 (17.9)	78 (16.5)	57 (13.3)	28 (20.3)
Stress					
Quartile 1	8 (22.2)	20 (17.7)	98 (24.2)	97 (26.4)	33 (30.8)
Quartile 2	10 (27.8)	26 (23.0)	95 (23.5)	102 (27.7)	25 (23.4)
Quartile 3	9 (25.0)	31 (27.4)	115 (28.4)	84 (22.8)	20 (18.7)
Quartile 4	9 (25.0)	36 (31.9)	97 (24.0)	85 (23.1)	29 (27.1)
Rotating Shift Work	16 (38.1)	19 (14.8)	69 (14.9)	67 (16.2)	36 (28.1)
Night Shift Work	17 (41.5)	31 (24.2)	97 (21.0)	83 (20.1)	46 (35.7)
Season					
Winter	7 (15.2)	29 (21.6)	102 (21.6)	98 (22.8)	41 (29.7)
Spring	12 (26.1)	34 (25.4)	152 (32.1)	115 (26.8)	35 (25.4)
Summer	14 (30.4)	34 (25.4)	99 (20.9)	96 (22.4)	34 (24.6)
Fall	13 (28.3)	37 (27.6)	120 (25.4)	120 (28.0)	28 (20.3)

Table 3.3. Association between sleep characteristics and live birth; EAGeR Study (2006-2012) (n=1088)[ab]

	Live Birth	Unadjusted			Multivariate adjusted[c]		
Sleep Characteristics	n (%)	RR	95% CI		RR	95% CI	
Sleep Duration							
Sleep <6 hours (n=38)	19 (50.0%)	0.90	0.57	1.42	0.98	0.61	1.57
Sleep 6-7 hours (n=115)	63 (54.8%)	0.96	0.73	1.26	0.99	0.75	1.32
Sleep 7-8 hours (n=432)	246 (56.9%)	Ref	--	--	Ref	--	--
Sleep 8-9 hours (n=393)	211 (53.7%)	0.93	0.77	1.42	0.89	0.74	1.07
Sleep ≥9 hours (n=107)	57 (53.3%)	0.93	0.70	1.24	0.89	0.66	1.19
Rotating Shift Work							
Not Rotating Shift Work (n=882)	479 (54.3%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=186)	110 (59.1%)	1.08	0.88	1.33	1.05	0.85	1.29
Night Shift Work							
Not Night Shift Work (n=817)	440 (53.9%)	Ref	--	--	Ref	--	--
Night Shift Work (n=249)	146 (58.6%)	1.09	0.91	1.31	1.07	0.88	1.30
Sleep Midpoint							
Sleep Midpoint Tertile 1 (n=370)	212 (57.3%)	1.01	0.83	1.22	1.03	0.85	1.26
Sleep Midpoint Tertile 2 (n=367)	206 (56.1%)	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=348)	178 (51.2%)	0.90	0.74	1.10	0.92	0.75	1.13
Social Jet Lag							
Social Jet Lag <1 hour (n=705)	397 (56.3%)	Ref	--	--	Ref	--	--
Social Jet Lag 1-2 hours (n=282)	151 (53.6%)	0.95	0.79	1.15	1.05	0.86	1.29
Social Jet Lag ≥2 hours (n=98)	48 (49.0%)	0.85	0.63	1.14	0.99	0.72	1.35

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]Weighted for loss-to-follow-up

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), and exercise (categorical; Low, Moderate, High)

Table 3.4. Association between sleep characteristics and any pregnancy loss among total pregnancies; EAGeR Study (2006-2012) (n=797)[ab]

	(n, %)	Unadjusted			Multivariate adjusted[c]		
	188	RR	95% CI		RR	95% CI	
Sleep Duration							
<7 hours (n=114)	29 (25.4%)	1.09	0.73	1.62	0.98	0.64	1.48
7-9 hours (n=600)	135 (22.5%)	Ref	--	--	Ref	--	--
≥9 hours (n=81)	23 (28.4%)	1.24	0.81	1.89	1.24	0.80	1.94
Rotating Shift Work							
Not Rotating Shift Work (n=640)	150 (23.4%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=144)	33 (22.9%)	0.96	0.66	1.39	0.99	0.67	1.46
Night Shift Work							
Not Night Shift Work (n=586)	137 (23.4%)	Ref	--	--	Ref	--	--
Night Shift Work (n=196)	47 (24.0%)	0.97	0.70	1.36	0.99	0.70	1.41
Sleep Midpoint							
Tertile 1 (n=277)	60 (21.7%)	1.01	0.71	1.43	0.98	0.69	1.40
Tertile 2 (n=272)	64 (23.5%)	Ref	--	--	Ref	--	--
Tertile 3 (n=246)	63 (25.6%)	1.13	0.81	1.59	1.06	0.74	1.51
Social Jet Lag							
Social Jet Lag <1 hour (n=526)	123 (23.4%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=269)	64 (23.8%)	1.07	0.80	1.43	0.93	0.68	1.28

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]Weighted for pregnancy and loss-to-follow-up

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), and exercise (categorical; Low, Moderate, High)

Table 3.5. Association between sleep characteristics and Adverse Outcomes of Placental Origin (Preterm Birth, Hypertensive Disorders of Pregnancy, and GDM); EAGeR Study (2006-2012) (n=598)[a]

	n (%)	Unadjusted			Multivariate Adjusted[b]		
Composite Outcome	118	RR	95% CI		RR	95% CI	
Sleep Duration							
Sleep <7 hours (n=81)	19 (23.5%)	1.15	0.72	1.86	1.28	0.76	2.13
Sleep 7-9 hours (n=460)	88 (19.1%)	Ref	--	--	Ref	--	--
Sleep ≥9 hours (n=56)	10 (17.9%)	0.88	0.49	1.59	1.06	0.56	2.00
Rotating Shift Work							
Not Rotating Shift Work (n=479)	98 (20.5%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=111)	17 (15.3%)	0.84	0.52	1.37	0.84	0.51	1.40
Night Shift Work							
Not Night Shift Work (n=440)	84 (19.1%)	Ref	--	--	Ref	--	--
Night Shift Work (n=147)	31 (21.1%)	1.04	0.69	1.58	1.09	0.71	1.70
Sleep Midpoint							
Sleep Midpoint Tertile 1 (n=213)	34 (16.0%)	0.64	0.41	1.01	0.62	0.39	0.99
Sleep Midpoint Tertile 2 (n=205)	44 (21.5%)	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=179)	39 (21.8%)	0.89	0.61	1.31	0.98	0.64	1.52
Social Jet Lag							
Social Jet Lag<1 hour (n=397)	64 (16.1%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=200)	53 (26.5%)	1.84	1.30	2.60	1.60	1.06	2.40
Continuous (n=597)	117 (19.6%)	1.08	0.96	1.23	1.01	0.86	1.18

[a]Imputed data for missing sleep and covariates were used in this analysis; weighted for pregnancies lasting ≥20 weeks, pregnancy, and lost-to-follow-up

[b]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), and exercise (Categorical; Low, Moderate, High)

Chapter 3 Supplementary Tables

Table 3.1S. Characteristics of participants (N=1220) by tertile of sleep midpoint; EAGeR Study (2006-2012)

Characteristics	Sleep Midpoint Tertile 1 n=406	Sleep Midpoint Tertile 2 n=404	Sleep Midpoint Tertile 3 n=410
	Mean (±SD)	Mean (±SD)	Mean (±SD)
Age (years)	30.1 (4.8)	29.1 (4.6)	27.2 (4.6)
BMI (kg/m^2)	26.5 (6.5)	26.1 (6.5)	26.4 (6.5)
Social Jet Lag (Minutes)**	30 (0, 60)	45 (4, 90)	65 (30, 108)
Sleep Midpoint (Hour:Min AM)**	2:45 AM (2:20 AM, 3:00 AM)	3:36 AM (3:25 AM, 3:48AM)	4:40 AM (4:21 AM, 5:17 AM)
Sleep Duration (Hours)**	7.92 (7.42, 8.40)	7.87 (7.33, 8.45)	8.00 (7.42, 8.72)
	n (%)	n (%)	n (%)
Sleep Duration			
<6 hours	12 (3.0)	13 (3.2)	21 (5.1)
6-7 hours	40 (9.9)	46 (11.4)	48 (11.7)
7-8 hours	186 (45.8)	161 (39.9)	129 (31.5)
8-9 hours	146 (36.0)	143 (35.4)	141 (34.4)
≥9 hours	22 (5.4)	41 (10.2)	71 (17.3)
Parity			
Nulliparous	155 (38.2)	162 (40.1)	204 (49.8)
1	150 (37.0)	148 (36.6)	133 (32.4)
2+	101 (24.9)	94 (23.3)	73 (17.8)
Marijuana Use	9 (2.2)	18 (4.5)	32 (7.8)
Opioid Use	19 (4.7)	26 (6.6)	40 (9.9)
Antidepressant Use	61 (15.0)	72 (17.8)	73 (17.8)
Sleep Aid Use	14 (3.5)	15 (3.8)	24 (5.9)
More than high school education	359 (88.4)	358 (88.6)	335 (81.9)
Race			
White	390 (96.1)	383 (94.8)	384 (93.7)
Cotinine (Smoking)	39 (9.7)	32 (8.1)	70 (17.3)
Alcohol consumption in past year			
Never	264 (65.5)	271 (67.6)	271 (66.4)
Sometimes	127 (31.5)	125 (31.2)	128 (31.4)
Often	12 (3.0)	5 (1.3)	9 (2.2)
Currently Employed	316 (79.6)	286 (73.7)	290 (73.6)
Caffeine Consumption			
Nondrinker	114 (28.1)	107 (26.5)	84 (20.5)

1-3 Cups/day	232 (57.1)	237 (58.7)	244 (59.5)
≥ 3 Cups/day	60 (14.8)	60 (14.9)	82 (20.0)
Stress			
Quartile 1	87 (24.4)	94 (27.7)	75 (22.5)
Quartile 2	93 (26.1)	77 (22.7)	88 (26.4)
Quartile 3	93 (26.1)	96 (28.3)	70 (21.0)
Quartile 4	83 (23.3)	72 (21.2)	101 (30.2)
Exercise			
Low	105 (25.9)	112 (27.7)	103 (25.1)
Moderate	170 (41.9)	162 (40.1)	164 (40.0)
High	131 (32.3)	130 (32.2)	143 (34.9)
Rotating Shift Work	57 (14.4)	66 (17.1)	84 (21.4)
Night Shift Work	72 (18.2)	86 (22.3)	116 (29.5)
Season			
Winter	94 (23.2)	101 (25.0)	82 (20.0)
Spring	119 (29.3)	104 (25.7)	125 (30.5)
Summer	83 (20.4)	88 (21.8)	106 (25.9)
Fall	110 (27.1)	111 (27.5)	97 (23.7)

** Median (Q1, Q3)

Table 3.2S. Association between sleep characteristics and hCG pregnancy loss among total pregnancies (n=797); EAGeR Study (2006-2012)[ab]

	n (%)	Unadjusted			Multivariate adjusted[c]		
	55	RR	95% CI		RR	95% CI	
Sleep Duration							
<7 hours (n=114)	10 (8.8%)	1.42	0.73	2.80	1.17	0.58	2.36
7-9 hours (n=600)	39 (6.5%)	Ref	--	--	Ref	--	--
≥9 hours (n=81)	6 (7.4%)	1.08	0.47	2.51	1.01	0.42	2.48
Rotating Shift Work							
Not Rotating Shift Work (n=640)	43 (6.7%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=144)	11 (7.6%)	1.16	0.60	2.24	1.19	0.60	2.34
Night Shift Work							
Not Night Shift Work (n=586)	38 (6.5%)	Ref	--	--	Ref	--	--
Night Shift Work (n=196)	17 (8.7%)	1.30	0.73	2.32	1.34	0.73	2.45
Sleep Midpoint							
Tertile 1 (n=277)	20 (7.2%)	1.56	0.78	3.10	1.49	0.74	3.01
Tertile 2 (n=272)	13 (4.8%)	Ref	--	--	Ref	--	--
Tertile 3 (n=246)	22 (8.9%)	1.71	0.87	3.35	1.54	0.76	3.11
Social Jet Lag							
Social Jet Lag <1 hour (n=526)	39 (7.4%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=269)	16 (5.9%)	0.78	0.44	1.36	0.62	0.33	1.14

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]Weighted for pregnancy and loss-to-follow-up

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (Categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (Categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), and exercise (Categorical; Low, Moderate, High)

Table 3.3S. Association between sleep characteristics and clinical pregnancy loss among clinically confirmed pregnancies; EAGeR Study (2006-2012) (n=732)[ab]

	(n, %)	Unadjusted			Multivariate adjusted[c]		
	133	RR	95% CI		RR	95% CI	
Sleep Duration							
<7 hours (n=101)	17 (16.8%)	0.88	0.53	1.44	0.85	0.50	1.44
7-9 hours (n=555)	93 (16.8%)	Ref	--	--	Ref	--	--
≥9 hours (n=74)	16 (21.6%)	1.27	0.79	2.02	1.24	0.75	2.05
Rotating Shift Work							
Not Rotating Shift Work (n=589)	102 (17.3%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=131)	21 (16.0%)	0.91	0.58	1.43	0.94	0.59	1.51
Night Shift Work							
Not Night Shift Work (n=542)	95 (17.5%)	Ref	--	--	Ref	--	--
Night Shift Work (n=175)	28 (16.0%)	0.81	0.53	1.25	0.85	0.55	1.33
Sleep Midpoint							
Tertile 1 (n=253)	37 (14.6%)	0.96	0.64	1.46	0.97	0.64	1.48
Tertile 2 (n=257)	49 (19.1%)	Ref	--	--	Ref	--	--
Tertile 3 (n=220)	40 (18.2%)	1.16	0.80	1.69	0.93	0.61	1.40
Social Jet Lag							
Social Jet Lag <1 hour (n=480)	80 (16.7%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=250)	46 (18.4%)	1.23	0.89	1.70	1.00	0.69	1.44

[a]Imputed data for missing sleep and covariates were used in this analysis

[b]Weighted for clinical confirmation of pregnancy, pregnancy, and loss-to-follow-up.

[c]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), and exercise (Categorical; Low, Moderate, High)

Table 3.4S. Association between sleep characteristics and Preterm Birth, Hypertensive Disorders of Pregnancy, and GDM; EAGeR Study (2006-2012) (n=598)[a]

	Total (%)	Unadjusted			Multivariate Adjusted[b]		
Preterm Birth	**52**						
Sleep Duration							
Sleep <7 hours (n=81)	13 (16.1%)	2.08	1.13	3.82	2.64	1.35	5.17
Sleep 7-9 hours (n=460)	34 (7.4%)	Ref	--	--	Ref	--	--
Sleep ≥9 hours (n=56)	5 (8.9%)	0.81	0.3	2.19	1.01	0.35	2.88
Rotating Shift Work							
Not Rotating Shift Work (n=479)	42 (8.8%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=111)	10 (9.0%)	1.13	0.58	2.19	1.05	0.51	2.13
Night Shift Work							
Not Night Shift Work (n=440)	35 (8.0%)	Ref	--	--	Ref	--	--
Night Shift Work (n=147)	17 (11.6%)	1.35	0.75	2.42	1.31	0.71	2.45
Sleep Midpoint							
Sleep Midpoint Tertile 1 (n=213)	14 (6.6%)	0.54	0.27	1.07	0.49	0.24	1.00
Sleep Midpoint Tertile 2 (n=205)	20 (9.8%)	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=179)	18 (10.1%)	0.74	0.41	1.34	0.99	0.52	1.88
Social Jet Lag							
Social Jet Lag<1 hour (n=397)	35 (8.8%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=200)	17 (8.5%)	1.10	0.65	1.86	1.01	0.55	1.85
Hypertensive Disorders of Pregnancy	**62**						
Sleep Duration							
Sleep <7 hours (n=81)	9 (11.1%)	1.21	0.64	2.29	1.09	0.54	2.21
Sleep 7-9 hours (n=460)	49 (10.7%)	Ref	--	--	Ref	--	--
Sleep ≥9 hours (n=56)	4 (6.5%)	0.49	0.17	1.40	0.68	0.23	2.02
Rotating Shift Work							
Not Rotating Shift Work (n=479)	52 (10.9%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=111)	8 (7.2%)	0.78	0.39	1.57	0.67	0.31	1.42
Night Shift Work							
Not Night Shift Work (n=440)	42 (9.6%)	Ref	--	--	Ref	--	--
Night Shift Work (n=147)	18 (12.2%)	1.46	0.85	2.51	1.57	0.88	2.81
Sleep Midpoint							
Sleep Midpoint Tertile 1 (n=213)	19 (8.9%)	0.76	0.41	1.41	0.66	0.35	1.26
Sleep Midpoint Tertile 2 (n=205)	23 (11.2%)	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=179)	20 (11.2%)	0.98	0.57	1.69	1.05	0.57	1.93
Social Jet Lag							
Social Jet Lag<1 hour (n=397)	30 (7.6%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=200)	32 (16.0%)	1.97	1.21	3.21	1.68	0.96	2.95
GDM	**22**						
Sleep Duration							

Sleep <7 hours (n=81)	2 (2.5%)	0.46	0.10	2.12	0.34	0.05	2.31
Sleep 7-9 hours (n=460)	18 (3.9%)	Ref	--	--	Ref	--	--
Sleep ≥9 hours (n=56)	2 (3.6%)	1.71	0.66	4.46	1.37	0.32	5.78
Rotating Shift Work							
Not Rotating Shift Work (n=479)	18 (3.8%)	Ref	--	--	Ref	--	--
Rotating Shift Work (n=111)	3 (2.7%)	1.18	0.46	3.06	1.14	0.36	3.65
Night Shift Work							
Not Night Shift Work (n=440)	17 (3.9%)	Ref	--	--	Ref	--	--
Night Shift Work (n=147)	4 (2.7%)	0.53	0.17	1.63	0.78	0.23	2.62
Sleep Midpoint							
Sleep Midpoint Tertile 1 (n=213)	7 (3.3%)	0.52	0.19	1.40	0.44	0.14	1.37
Sleep Midpoint Tertile 2 (n=205)	9 (4.4%)	Ref	--	--	Ref	--	--
Sleep Midpoint Tertile 3 (n=179)	6 (3.4%)	0.74	0.32	1.7	0.69	0.22	2.13
Social Jet Lag							
Social Jet Lag<1 hour (n=397)	9 (2.3%)	Ref	--	--	Ref	--	--
Social Jet Lag ≥1 hours (n=200)	13 (6.5%)	3.60	1.56	8.32	3.63	1.23	10.7

[a]Imputed data for missing sleep and covariates were used in this analysis; weighted for clinically recognized pregnancies lasting ≥20 weeks, pregnancy, and lost-to-follow-up

[b]Adjusted for: age (continuous; years), BMI (continuous; kg/m^2), parity (categorical; Nulliparous, 1, ≥2), stress (quartiles; No stress, Little stress, Moderate stress, A lot of stress), opioid use (binary; Yes, No), marijuana use (binary; Yes, No), antidepressant use (binary; Yes, No), race (binary; White, Non-white), education (binary; <High School, ≥High School), smoking (binary; Yes, No), employed (binary; Yes, No), alcohol (binary; Yes, No), caffeine (categorical; Nondrinker, 1-3 cups/day, >3 cups/day), season (categorical; Fall, Winter, Spring, Summer), sleep aid use (binary; Yes, No), and exercise (categorical; Low, Moderate, High)

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