HHS Public Access

Author manuscript

Chronobiol Int. Author manuscript; available in PMC 2021 February 01.

Published in final edited form as:

Chronobiol Int. 2020 February ; 37(2): 260–271. doi:10.1080/07420528.2019.1694938.

The influences of sleep duration, chronotype, and nightwork on the ovarian cycle

Kara A. Michels[a], **Pauline Mendola**[a], **Karen C. Schliep**[b], **Edwina H. Yeung**[a], **Aijun Ye**[a], **Galit L. Dunietz**[c], **Jean Wactawski-Wende**[d], **Keewan Kim**[a], **Joshua R. Freeman**[a], **Enrique F. Schisterman**[a], **Sunni L. Mumford**[a]

[a]Epidemiology Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda MD

[b]Department of Family and Preventive Medicine, Division of Public Health, University of Utah School of Medicine, Salt Lake City UT

[c]Division of Sleep Medicine, Department of Neurology, University of Michigan, Ann Arbor MI

[d]Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University at Buffalo, The State University of New York, Buffalo NY

Abstract

Despite research indicating that sleep disorders influence reproductive health, the effects of sleep on reproductive hormone concentrations are poorly characterized. We prospectively followed 259 regularly menstruating women across one to two menstrual cycles (the BioCycle Study, 2005–2007), measuring fasting serum hormone concentrations up to eight times per cycle. Women provided information about daily sleep in diaries and chronotype and night/shift work on a baseline questionnaire. We evaluated percent differences in mean hormone concentrations, the magnitude of shifts in the timing and amplitude of hormone peaks, and the risk for sporadic anovulation associated with self-reported sleep patterns and night/shift work. We estimated chronotype scores—categorizing women below and above the interquartile range (IQR) as "morning" and "evening" chronotypes, respectively. For every hour increase in daily sleep duration, mean estradiol concentrations increased by 3.9% (95% confidence interval [CI] 2.0, 5.9%) and luteal phase progesterone by 9.4% (CI 4.0, 15.2%). Receiving less than 7 hours of sleep per day was associated with slightly earlier rises in peak levels for several hormones. Women reporting night/shift work (n=77) had lower testosterone relative to women employed without night/shift work (percent difference: −9.9%, CI −18.4, −0.4%). Women with morning chronotypes (n=47) had earlier rises in estradiol during their cycles and potentially an earlier rise in luteinizing

Corresponding author: Sunni L. Mumford, Ph.D, Earl Stadtman Investigator, Epidemiology Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, 6710B Rockledge Drive MSC 7004, Bethesda, MD 20892, Tel: 301-435-6946, mumfords@mail.nih.gov.

Disclosures of Interest

The authors report no conflict of interest.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

hormone. Compared to those who had intermediate chronotypes, women with evening chronotypes (n=42) had a later luteinizing hormone peak of borderline statistical significance. A reduced risk for sporadic anovulation was suggested, but imprecise, for increasing hours of daily sleep leading up to ovulation (risk ratio 0.79, CI 0.59, 1.06), while an imprecise increased risk was observed for women with morning chronotypes (risk ratio 2.50, CI 0.93, 6.77). Sleep-related hormonal changes may not greatly alter ovarian function in healthy women, but have the potential to influence gynecologic health.

Keywords

sleep; menstrual cycle; hormones; anovulation; longitudinal studies

Introduction

The American Academy of Sleep Medicine, the Sleep Research Society, and the National Sleep Foundation recommend that adults sleep at least 7 hours per night on a regular basis (Watson et al., 2015; Hirshkowitz et al., 2015). However, data from large surveys indicate that as many as a third of women in the United States report sleeping less than 6 to 7 hours in a typical 24 hour period (Centers for Disease Control and Prevention, 2008, 2011). In a recent meta-analysis, short sleep duration was associated with increased risk of mortality and chronic diseases (Itani et al., 2016). Scientific reviews highlight the potential for sleep disorders or changes in sleep duration and timing to alter aspects of reproductive health such as fertility (Kloss et al., 2015), but biologic mechanisms are unclear. The circadian system is known to influence cholesterol synthesis pathways, which precede sex steroid hormone production (Panda, 2016; Urlep & Rozman, 2013). The suprachiasmatic nucleus, which governs circadian rhythmicity, has sex steroid hormone receptors; both animal and human studies indicate that feedback mechanisms between circadian rhythms, these hormones, and gonadotropins exist (reviewed in Mong et al., 2011; Lord et al., 2014; Rossmanith, 1998). Furthermore, light exposure and changes in melatonin levels are thought to influence risk for menstrual cycle disorders; melatonin may help regulate oxidative stress within the ovary and melatonin receptors help control the release of gonadotropins (Barron, 2007; Reiter et al., 2014; Reiter et al., 2009).

Among reproductive-aged women, complex feedback mechanisms regulate sex steroid hormone and gonadotropin levels across the menstrual cycle. As such, there have been studies of sleep quality across the cycle, but associations between sleep behaviors and hormone concentrations among reproductive-aged women are not well characterized (Lord et al., 2014). Studies exploring sleep patterns across the menstrual cycle tend to assess sleep quality in small groups of women under experimental conditions; focus on women with conditions such as premenstrual dysphoric disorder and polycystic ovarian syndrome; or if hormone concentrations are assayed, rely on only one or two measurements within a menstrual cycle (Lord et al., 2014; Driver & Baker, 1998; Baker & Lee, 2018; Sun et al., 2019; Shechter et al., 2010; de Zambotti et al., 2015; Driver et al., 1996; Sharkey et al., 2014). Additionally, few studies evaluate hormone concentrations or indicators of ovarian cycle functioning in the context of sleep characteristics such as chronotype. Chronotype is a

measure of one's preference for the timing of sleep and activity (i.e., morningness or eveningness) and it is influenced by both circadian rhythms, age, and social factors (Roenneberg et al., 2007). Chronotype can account for not only sleep duration, but also for changes in the duration and timing of sleep across weekdays and weekends.

To better understand the connections between sleep and the ovarian cycle, we used data from a group of healthy, regularly menstruating women who were followed across two menstrual cycles. Women provided multiple serum samples across their cycles and completed questionnaires and daily diaries. This longitudinal data collection facilitated our assessing differences in hormone concentrations, the timing (i.e., phasing) and amplitude of hormone peaks across the cycle, and the risk for sporadic anovulation associated with self-reported sleep patterns and characteristics (daily hours of sleep, chronotype, night or shift work).

Materials and Methods

Study population

The BioCycle Study (2005–2007) is a prospective cohort of 259 women recruited from upstate New York (NY), U.S. These women were healthy, regularly menstruating, and aged 18–44 years. Women were excluded if they were currently using oral contraceptives or had used them within the last three months; were currently using other medications or vitamins; if they had been pregnant in the last six months; or if they had any diagnoses of certain chronic conditions (e.g., menstrual and ovulation disorders, fibroids, history of cancer). Women should not have had gynecologic surgeries in the past year, but it is possible that a woman could enroll if she previously had a unilateral oophorectomy and was still regularly menstruating, but this information was not collected. Further details of the study design and exclusion criteria have been published (Wactawski-Wende et al., 2009).

Ethical approval

The University at Buffalo Health Sciences Institutional Review Board (IRB) approved study procedures and served as the IRB designated by the National Institutes of Health under a reliance agreement. All participants provided written informed consent.

Data collection

Participants were followed for one (n=9) to two (n=250) menstrual cycles. Fasting blood samples were collected at eight clinic visits across each menstrual cycle (up to sixteen samples per participant): on the second day of menstruation (1 visit), during the mid- and late follicular phase (2 visits), on the day of the luteinizing hormone (LH) peak (1 visit), on the day of predicted ovulation (1 visit), and during the early, mid, and late luteal phase (3 visits). Visits were scheduled to occur in the morning to reduce diurnal variation. Fertility monitors (Clearblue Easy Fertility Monitor; Inverness Medical, Waltham, Massachusetts [MA]) were used to assist in the timing of mid-cycle clinic visits (Howards et al., 2009). Ninety-four percent of participants completed at least seven clinic visits per cycle. Most women participated in the study for two consecutive menstrual cycles (91%). Of the 24 cycles [9%] that were not consecutive, the median interval between cycles was 30 days (IQR

27 to 45; range 19 to 143 days). Prior to hormone measurement, specimens were processed and frozen at −80°C within 90 minutes of blood draw.

Participants completed baseline questionnaires and provided information on demographics and their lifestyles and health histories. In each menstrual cycle, participants also completed up to four 24-hour dietary recalls; from this, we estimated average daily caloric intake and caffeine intake, as well as the percent of the diet attributable to macronutrients (i.e., fat, protein, carbohydrates) and alcohol, for each “phase” within each ovarian cycle (follicular, ovulatory, or luteal). Dietary questionnaire data were analyzed with the Nutrition Data System for Research software (version 2005) developed by the Nutrition Coordinating Center of the University of Minnesota (Minneapolis, Minnesota). Participants completed daily diaries, which included questions on daily sleep duration (defined below) and cigarette smoking (defined as any cigarette use in the two days prior to each clinic visit compared to none). Study personnel used calibrated scales and standardized procedures to measure weight and height (for calculating body mass index [BMI]).

Assessment of sleep habits

Our exposures of interest were daily sleep duration, night or shift work, and chronotype. To estimate daily sleep duration, we used information from diaries. Each day, participants reported their total hours of sleep from the 24 hours prior, including nightly sleep and naps, but they did not report the timing of their sleep. To create a measure of sleep duration around the time of hormone measurement, average daily sleep duration was defined as the average hours of sleep on the day of and the day before the clinic visits (a continuous variable that varied across each menstrual cycle for each woman). The median hours of sleep per day calculated from all of these two-day averages (all women, all clinic visits) was 7 hours (interquartile range: 6.3, 8.0). Accordingly, we created a dichotomous variable reflecting whether the average daily sleep at each clinic visit was <7 hours or ≥7 hours (reference). Missingness of the diary-based sleep information was generally low (<15 women per visit), but n=19 and n=27 women did not provide diary-based information on the last late luteal phase visit in cycle 1 and 2, respectively.

At baseline, participants were asked if they were currently employed and if they worked nights or rotating shifts. From this, we created a categorical variable: unemployed, employed without night or shift work (reference), and employed with night or shift work. Thirty-five women were missing employment information.

Since timing of sleep (i.e., time of sleep onset and waking) was unavailable in the diaries and daily sleep duration included naps, we did not use diary information to estimate chronotype. On the baseline questionnaire, participants reported their usual bedtimes, the amount of time they typically take to fall asleep, and the usual time they get up in the mornings on both weekdays and free days. Using this information, we estimated sleep midpoints across the week and generated chronotype scores as described by Roenneberg and colleagues: a measure of an individual’s natural midpoint of sleep on free days with correction for their sleep debt accumulated during the work week (i.e., a lack of sleep on work nights) (Roenneberg et al., 2004; Roenneberg & Merrow, 2007). To contrast women with scores indicating a strong “morningness” preference and women with a preference for

"eveningness" to those without a clear preference for either, we categorized chronotype using the interquartile range of these scores. Women with scores of 2.0 to <3.5 were grouped as "morning" chronotypes and women with scores >6.0 had "evening" chronotypes. For brevity, we refer to women with chronotype scores within the interquartile range as having "intermediate" chronotypes (including the 25th and 75th percentiles). Fourteen women were missing chronotype information. Unlike daily sleep, each participant was assigned one chronotype and night/shift work status; these exposures were fixed for all time points/clinic visits and did not vary across the menstrual cycle.

Reproductive hormone measurement

Total estradiol, follicle stimulating hormone (FSH), LH, and progesterone were measured from fasting serum at the Kaleida Health Center for Laboratory Medicine using solid-phase competitive chemiluminescent enzymatic immunoassays (Specialty Laboratories Inc., Valencia, California) on a DPC Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, Illinois). Total testosterone was measured in frozen stored samples by liquid chromatography/tandem mass spectrometry employing a Shimadzu Prominence Liquid Chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) with an ABSceix 5500 tandem mass spectrometer (AB SCIEX, Framingham, Massachusetts) at the University of Minnesota. Increased sensitivity was obtained by using Mobile Phase B (100% acetonitrile) with a low standard of 4 ng/dL added to the standard curve. The coefficients of variation for these tests reported by the laboratories were <10% for estradiol, <5% for LH and FSH, <14% for progesterone, and <7% for total testosterone. The number of measurements missing ranged from 4–5% for all the hormones.

For our analyses on anovulation, cycles with progesterone concentrations ≤5 ng/mL and no observed serum LH peak in the mid or late luteal phase samples (in the event that timing of progesterone measurements was too early) were considered anovulatory (n=42) (Lynch et al., 2014).

Statistical analyses

Descriptive statistics for our population are presented in Table 1. To visualize overall sleep patterns across the menstrual cycle, we used linear mixed models to estimate unadjusted population least-square means for the average daily sleep durations at each clinic visit (using data from both cycles) (Supplemental Figure 1).

Models for hormone differences associated with sleep

We ran linear mixed models with random intercepts ('proc mixed' modeling in SAS) and inverse probability weighting to model the percent difference in mean hormone concentrations associated with each sleep exposure (daily hours of sleep, night/shift work, chronotype). Hormones were log-transformed to achieve normality and effect estimates were back-transformed and interpreted as percent difference. These longitudinal models used data from each clinic visit across both cycles and accounted for repeated observations (up to eight clinic visits per menstrual cycle and up to two cycles per woman). For progesterone, we limited the models to those observations from the luteal phase in each of the cycles, as we expected low concentrations and little variation in the follicular phase. Similarly, models for

LH and FSH were limited to the observations around expected ovulation—those corresponding to the late follicular phase, LH peak, and predicted ovulation. For FSH, we also report estimates including all visits, as FSH concentrations slightly increase at the end of the luteal phase.

In these analyses, we treat sleep characteristics as our exposures. However, hormone concentrations on a given clinic visit potentially affect sleep behaviors at the next visit as well (i.e., both confounding and mediating relationships exist). We accounted for this time-varying confounding between daily sleep and hormones across the menstrual cycle by generating inverse probability weights for each clinic visit in each cycle. These weights incorporated information on hormone levels and in the models for sleep duration, information on sleep duration from the prior clinic visits (Cole & Hernan, 2008; Moodie & Stephens, 2011). All hormones except testosterone were included in the weights, which would not be expected to greatly influence hormonal feedback for other hormones. The weights also accounted for other confounders that do not vary across the menstrual cycle (see below). The inverse probability weights for the night/shift work and chronotype models also control for the cyclic variation in hormones across the cycle, but not sleep duration. Chronotype and night/shift work status were established at baseline, before our measurement of daily sleep during the cycles; therefore, we did not adjust for a potential causal mechanism through which these exposures may influence hormone levels.

Selection of confounders for the weighted models

Potential confounders were selected *a priori* from the literature and with the use of causal diagrams. Similar to the complicated relationship between sleep and hormone levels, we recognize that 1) dietary intake can influence both daily sleep and hormone levels and 2) daily sleep can also influence one's diet (e.g., caffeine or carbohydrate consumption) (St-Onge et al., 2016; Peuhkuri et al., 2012; Dashti et al., 2015). Therefore, in the inverse probability weighted models for daily sleep, the weights controlled for time-varying confounding by both changing hormone levels and changing diet—which we allowed to vary by "phase" in each cycle (follicular, ovulatory, luteal). We also used the weights to control for the season of each menstrual cycle. Though an understudied topic in humans, studies from Norway and Russia note seasonal variation in sex steroid hormone and gonadotropin levels among reproductive aged women (Bjornerem et al., 2006; Kauppila et al., 1987; Danilenko et al., 2011). This may be due to changes in light exposure and corresponding changes in melatonin or vitamin D availability, both of which play a role in ovarian functioning (Barron, 2007; Irani & Merhi, 2014). We assigned a season to each menstrual cycle based on the date of the LH surge (December-February=winter, March-May=spring, June-August=summer, September-November=fall). Most of the cycles were in the same or similar seasons (e.g., end of spring, then beginning of summer); only 22 women had cycles in different seasons.

The weighted models for daily sleep duration were adjusted for age, race, BMI, chronotype, night/shift work status, average hours of sleep per day from the prior clinic visit, hormone levels at the prior clinic visit (estradiol, progesterone, FSH, and LH), average daily caloric intake, average daily caffeine intake, cigarette use, season, and the percent of energy intake

attributable to alcohol, fat, protein, and carbohydrates. Weighted models for night/shift work status and chronotype were adjusted for: age, race, BMI, chronotype (night/shift work models), night/shift work status (chronotype models), season, and hormone levels from the prior clinic visits. We chose not to adjust for factors such as diet in models for these sleep characteristics because night/shift work and chronotype were established at baseline, before we assessed diet during each of the menstrual cycles. Diet may be a mechanism through which night/shift work or chronotype affect hormone metabolism—several groups note that chronotype is associated with dietary habits (Lucassen et al., 2013; Maukonen et al., 2016, Sato-Mito et al., 2011).

Models to assess shifts in hormone peaks across the cycle

To further assess associations between sleep characteristics and hormonal patterns, we also estimated non-linear mixed models with harmonic terms. This unique modeling approach takes into account between- and/or within-subject variation and allows for estimation of mean concentrations over the entire menstrual cycle (similar to the linear mixed model approach described above, but data from all clinic visits were used for all hormones), as well as the estimation of amplitude (i.e., difference between nadir and peak concentrations) and timing of hormone peaks/phase shifts (Albert & Hunsberger, 2005). Given that the magnitude and timing of hormone peaks are relevant for understanding menstrual cycle fluctuations, these models offer important insight into potential associations between sleep and hormonal patterns. In these models, the cycle timescale was centered on ovulation and scaled to the observed cycle length (i.e., first cycle day=0, last cycle day=1, and predicted ovulation day=0.5). The use of cycle time centered on ovulation accommodates variability in follicular and luteal phase lengths across the cycles and allows for appropriate cycle phase comparisons of hormone concentrations and peaks. Our covariate adjustment for these models was similar to that used for the linear mixed modeling, though these models do not use weighting to adjust for cycle phase.

Models for estimating risk of sporadic anovulation

We used modified Poisson regression with robust errors to obtain risk ratios (RR) and 95% confidence intervals (CI) for the risk of sporadic anovulation associated with our sleep exposures. These models accounted for multiple cycles per woman (up to two) and use longitudinal modeling, but not inverse probability weighting. For daily sleep, we used the average daily sleep leading up to the predicted LH surge as the exposure in each menstrual cycle. These models were similarly adjusted for age, race, BMI, night/shift work status, chronotype, season; and from the LH surge in each cycle, average daily caloric intake, average daily caffeine intake, cigarette use, and the percent of energy intake attributable to alcohol, fat, protein, and carbohydrates. Models for night/shift work status and chronotype were adjusted for age, race, BMI, chronotype (night/shift work models), night/shift work status (chronotype models), and season. We did not adjust for hormone concentrations in any of the sporadic anovulation models because they are used to define the anovulation outcome in each cycle. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

Daily sleep duration

Women reported shorter daily sleep durations at ovulation and their longest sleep durations in the mid-luteal phase (Supplemental Figure S1); the difference between these two time points reached statistical significance, while other comparisons across the cycle did not (Bonferroni corrected, p=0.02). The least-square means ranged from 6.9 to 7.1 hours of daily sleep across the cycle.

We noted increases in both estradiol and luteal phase progesterone as average hours of daily sleep increased (Table 2). For every hour increase, we noted mean estradiol levels increased by 3.9% (CI 2.0, 5.9%) and mean luteal progesterone levels increased by 9.4% (CI 4.0, 15.2%). Correspondingly, reductions in mean estradiol (−7.0% CI −11.3, −2.5%) and luteal phase progesterone (−9.5% CI −20.2, 2.6%) were indicated for women reporting <7 hours sleep per day leading up to the clinic visits, but null changes could not be ruled out for the latter. Potential differences in FSH associated with sleep duration were indicated, but not precise. In our harmonic models (Supplemental Materials: Table S1, Figures S2–S4), women reporting <7 hours of daily sleep did have slightly lower mean estradiol levels. These women also had lower LH levels across the cycle—which was not detected in the weighted mixed models that limited observations to those around ovulation. Reporting average daily sleep of <7 hours was associated with earlier phase shifts (i.e., peaks) for LH, FSH, and progesterone, and we noted an earlier phase shift of borderline statistical significance for estradiol. A decreased risk for sporadic anovulation was suggested with increasing hours of daily sleep leading up to the LH surge, but did not reach statistical significance (RR 0.79, CI 0.59, 1.06). Sleeping <7 hours per day around the LH surge, did not translate into an increased risk for sporadic anovulation (Table 3).

Night/Shift work

Women reporting employment with night/shift work (n=77) or unemployment (n=44) had significantly lower mean concentrations of testosterone relative to women who were employed without night/shift work (night/shift work percent difference: −9.9%, CI −18.4, −0.4%; unemployed percent difference: −12.0%, 95% CI −21.4, −1.4%; Table 2). In our harmonic models (Table S2; Figures S5–S6), we observed lower mean testosterone levels across the cycle for unemployed women; lower levels were suggested, but imprecise, for those employed with night/shift work. Women reporting night/shift work had higher mean LH concentrations across the entire menstrual cycle in the harmonic models. Employment type was not associated with sporadic anovulation (Table 3).

Chronotype

We did not identify differences in mean hormone concentrations associated with chronotype in the weighted linear mixed models (Table 2). However, in the harmonic models (Table S3; Figures S7–S9), we noted an earlier phase shift in estradiol and a suggested, but not statistically significant, earlier rise in LH for women with morning chronotypes. Among women with evening chronotypes, a lower peak amplitude for progesterone and later rise in LH was suggested. Women reporting evening chronotypes did not have increased risk for

sporadic anovulation compared to those with an intermediate chronotype, but an increased risk for those with morning chronotypes was suggested (RR 2.50, CI 0.93, 6.77) (Table 3).

Discussion

Among our study population of regularly menstruating women, we observed that sleep patterns changed over the menstrual cycle—with the shortest sleep durations reported around ovulation. We were able to evaluate multiple sleep exposures in the same study and interestingly, each sleep characteristic was associated with unique differences in hormone levels. Estradiol and progesterone were altered with sleep duration, but not with exposures strongly associated with sleep timing: night/shift work and chronotype. All sleep characteristics that we evaluated were associated with only small shifts in the timing of events during the menstrual cycle (i.e., hormone peaks). None of these hormonal changes translated into clear differences in risk for sporadic anovulation. These results suggest that sleep characteristics among a group of healthy, reproductive-aged women may not greatly impact ovarian function, but they do have the potential to influence health through sex steroid hormone and gonadotropin metabolism. Sleep duration and associated behaviors (e.g., diet and eating times) can also alter risk for reproductive health problems through other aspects of lipid and energy metabolism (Kloss et al., 2015; Panda, 2016, Urlep & Rozman, 2013).

The differences in the timing of hormone peaks across the menstrual cycle that we noted with some sleep characteristics were often only shifts in fractions of a day. Therefore, these shifts may not have great clinical implications. However, in population-based studies—especially those with few hormone measurements—one can easily understand how these shifts could lead to erroneous conclusions about differences in hormone concentrations between groups with varying sleep habits. We also show “suggested” hormone shifts for some sleep exposures (our supplemental figures). Although effect estimates may not have reached statistical significance when comparing to reference groups, we observed larger and potentially biologically meaningful shifts when visualizing the hormone patterns for all levels of a sleep exposure together. For example: the LH peaks appear to differ by a day when contrasting the morning and evening chronotype groups with each other, rather than with the intermediate chronotype group.

Our unique longitudinal data and modeling approaches make it difficult to compare findings with prior studies. We believe Touzet and colleagues conducted the only other study that used linear mixed modeling and data from multiple hormone assessments across menstrual cycles, though they measured different hormones in urine. They noted a positive correlation between FSH and sleep duration (Touzet et al., 2002). The changes in estradiol and progesterone that we found with sleep duration correspond with women reporting less sleep around ovulation, when concentrations of these hormones are lowest.

Night/shift work is associated with exposure to light at night and circadian misalignment of sleep/wake and dietary/metabolic rhythms. Physiologically, shift work has been linked to alterations in DNA methylation (Bhatti et al., 2015; Zhu et al., 2011), but circadian disruption may impact glucose metabolism, inflammation, and oxidative stress—all of

which play a role in the ovarian cycle (Kecklund & Axelsson, 2016). We know the circadian clock is important in pregnancy and parturition (reviewed in Gamble et al., 2013), but the relationships between night/shift work and reproductive hormone levels are uncertain. Researchers for the Nurses' Health Study identified lower geometric mean levels of estradiol among premenopausal women who never worked night shifts, compared to women who worked 15 or more years of night shifts (Schernhammer et al., 2004). These researchers did not see differences in testosterone concentrations, but the androgen precursor dehydroepiandrosterone (DHEA), DHEA-sulfate, and androstenedione (which can be converted to/from testosterone) were lower among those who worked night shifts (Schernhammer et al., 2004). Two other studies reported higher estradiol concentrations among shift workers (Bracci et al., 2014; Gomez-Acebo et al., 2015). In our analyses, reproductive-aged women who were unemployed and those working nights/shifts had lower concentrations of testosterone. The women who were unemployed likely represent a heterogeneous or unique group—such as students. We also saw that night/shift work was associated with higher LH levels across the cycle. A Seattle based study found higher FSH and LH levels in the luteal phase among shift workers (Davis et al., 2012).

Few studies explore chronotype and reproduction, but there is increasing interest in this measure because it accounts for how long a person sleeps and when they sleep. "Evening" types tend to go to bed later on weekdays and sleep longer on weekends relative to those with intermediate or early chronotypes (Roenneberg et al., 2007). Chronotype is affected by both genetic and social factors (e.g., work schedules) and is associated with smoking, diet, and obesity—all of which influence reproductive function (Wittmann et al., 2006; Lucassen et al., 2013; Maukonen et al., 2016; Sato-Mito et al., 2011). Bracci and colleagues report a positive correlation between chronotype (i.e., increasing eveningness) and estradiol concentrations, though women in this study were older than those enrolled in the BioCycle Study and the researchers measured estradiol once during the follicular phase (Bracci et al., 2014).

To our knowledge, ours is the first study to assess changes in hormone concentrations across the menstrual cycle by chronotype. We were able to calculate this metric with information reported by participants at baseline, but we could not use our questionnaire data to differentiate between waking and rising in the mornings, nor did we have a validated sleep questionnaire designed to assess chronotype. We also did not have information about night and shift work occurring during each menstrual cycle and we may be underestimating the effect that this exposure has on hormone levels. However, a strength of our study is the prospective assessment of multiple measures of sleep behavior in the same population. Our daily sleep estimates were based on diary information recalled by participants during the menstrual cycle, which potentially reduces measurement error when compared to a single report of typical sleep.

The variability in both sleep and hormone levels across the menstrual cycle indicates that our use of repeated measurements and longitudinal modeling is warranted. Uniquely, we incorporated harmonic modeling that allowed us to evaluate hormone peak amplitudes and phase shifts across the cycles. Examination of too few time points and not controlling for variability in hormone levels could lead to incorrect conclusions about relative

concentrations of hormones between women with and without certain sleep behaviors. While we adjusted for hormone levels and sleep duration to control for variability by phase, we are not able to rule out reverse causality. There is potential for sleep duration to alter reproductive hormone levels and these hormones may also influence sleep duration. For example, sleep disruption and insomnia are commonly reported during the menopausal transition, which in part, is attributable to the cessation of estrogen production (Monteleone et al., 2018).

Ultimately, to better understand the short- and long-term effects of sleep duration on reproductive hormone metabolism, our findings should be replicated in other populations of healthy women with detailed occupational and sleep information, as well as within groups of women at greater risk for anovulation and infertility (e.g., among women with higher BMIs or disorders such as polycystic ovarian syndrome). The BioCycle Study selection criteria led to the exclusion of women with these characteristics.

In our study, sleep characteristics among healthy, reproductive-aged women did not greatly alter ovarian function. However, we demonstrated a potential for sleep characteristics to influence reproductive hormone concentrations and the timing of hormone peaks. While none of the hormonal changes we observed clearly translated into increased risk for sporadic anovulation, the suggested associations with sleep duration and morning chronotype merit further investigation. The long-term influence of chronotype and night/shift work also need evaluation due to the increasing evidence that these exposures influence obesity and behavioral factors that are associated with infertility and increased risks for many chronic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank the BioCycle Study participants for their time and efforts.

Funding

This work was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (Contract Numbers: HHSN275200403394C, HHSN275201100002I, and Task 1 HHSN27500001).

References

Albert PS, & Hunsberger S (2005). On analyzing circadian rhythms data using nonlinear mixed models with harmonic terms. Biometrics, 61(4), 1115–1120; discussion 1120–1112. doi:10.1111/j.0006-341X.2005.464_1.x [PubMed: 16401286]

Baker FC, & Lee KA (2018). Menstrual Cycle Effects on Sleep. Sleep Medicine Clinics, 13(3), 283–294. doi:10.1016/j.jsmc.2018.04.002 [PubMed: 30098748]

Barron ML (2007). Light exposure, melatonin secretion, and menstrual cycle parameters: an integrative review. Biological Research for Nursing, 9(1), 49–69. doi:10.1177/1099800407303337 [PubMed: 17601857]

Bhatti P, Zhang Y, Song X, Makar KW, Sather CL, Kelsey KT, et al. (2015). Nightshift work and genome-wide DNA methylation. Chronobiology International, 32(1), 103–112. doi:10.3109/07420528.2014.956362 [PubMed: 25187986]

Bjornerem A, Straume B, Oian P, & Berntsen GK (2006). Seasonal variation of estradiol, follicle stimulating hormone, and dehydroepiandrosterone sulfate in women and men. Journal of Clinical Endocrinology and Metabolism, 91(10), 3798–3802. doi:10.1210/jc.2006-0866 [PubMed: 16835279]

Bracci M, Manzella N, Copertaro A, Staffolani S, Strafella E, Barbaresi M, et al. (2014). Rotating-shift nurses after a day off: peripheral clock gene expression, urinary melatonin, and serum 17-beta-estradiol levels. Scandinavian Journal of Work, Environment and Health, 40(3), 295–304. doi:10.5271/sjweh.3414

Centers for Disease Control and Prevention. (2008). QuickStats: Percentage of Adults Aged > 18 Years Who Reported an Average of < 6 Hours of Sleep per 24-Hour Period, by Sex and Age Group --- National Health Interview Survey, United States, 1985 and 2006. MMWR, 57(08), 209 https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5708a8.htm

Centers for Disease Control and Prevention. (2011). Unhealthy Sleep-Related Behaviors --- 12 States, 2009. MMWR, 60(08), 233–238. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6008a2.htm [PubMed: 21368738]

Cole SR, & Hernan MA (2008). Constructing inverse probability weights for marginal structural models. American Journal of Epidemiology, 168(6), 656–664. doi:10.1093/aje/kwn164 [PubMed: 18682488]

Danilenko KV, Sergeeva OY, & Verevkin EG (2011). Menstrual cycles are influenced by sunshine. Gynecological Endocrinology, 27(9), 711–716. doi:10.3109/09513590.2010.521266 [PubMed: 20937003]

Dashti HS, Scheer FA, Jacques PF, Lamon-Fava S, & Ordovas JM (2015). Short sleep duration and dietary intake: epidemiologic evidence, mechanisms, and health implications. Advances in Nutrition, 6(6), 648–659. doi:10.3945/an.115.008623 [PubMed: 26567190]

Davis S, Mirick DK, Chen C, & Stanczyk FZ (2012). Night shift work and hormone levels in women. Cancer Epidemiology, Biomarkers and Prevention, 21(4), 609–618. doi:10.1158/1055-9965.Epi-11-1128

de Zambotti M, Willoughby AR, Sassoon SA, Colrain IM, & Baker FC (2015). Menstrual Cycle-Related Variation in Physiological Sleep in Women in the Early Menopausal Transition. Journal of Clinical Endocrinology and Metabolism, 100(8), 2918–2926. doi:10.1210/jc.2015-1844 [PubMed: 26079775]

Driver HS, & Baker FC (1998). Menstrual factors in sleep. Sleep Medicine Reviews, 2(4), 213–229. [PubMed: 15310493]

Driver HS, Dijk DJ, Werth E, Biedermann K, & Borbely AA (1996). Sleep and the sleep electroencephalogram across the menstrual cycle in young healthy women. Journal of Clinical Endocrinology and Metabolism, 81(2), 728–735. doi:10.1210/jcem.81.2.8636295 [PubMed: 8636295]

Gamble KL, Resuehr D, & Johnson CH (2013). Shift work and circadian dysregulation of reproduction. Frontiers in Endocrinology, 4, 92. doi:10.3389/fendo.2013.00092 [PubMed: 23966978]

Gomez-Acebo I, Dierssen-Sotos T, Papantoniou K, Garcia-Unzueta MT, Santos-Benito MF, & Llorca J (2015). Association between exposure to rotating night shift versus day shift using levels of 6-sulfatoxymelatonin and cortisol and other sex hormones in women. Chronobiology International, 32(1), 128–135. doi:10.3109/07420528.2014.958494 [PubMed: 25216206]

Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. (2015). National Sleep Foundation's sleep time duration recommendations: methodology and results summary. Sleep Health, 1(1), 40–43. doi:10.1016/j.sleh.2014.12.010 [PubMed: 29073412]

Howards PP, Schisterman EF, Wactawski-Wende J, Reschke JE, Frazer AA, & Hovey KM (2009). Timing clinic visits to phases of the menstrual cycle by using a fertility monitor: the BioCycle Study. American Journal of Epidemiology, 169(1), 105–112. doi:10.1093/aje/kwn287 [PubMed: 18974081]

Irani M, & Merhi Z (2014). Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertility and Sterility, 102(2), 460–468.e463. doi:10.1016/j.fertnstert.2014.04.046 [PubMed: 24933120]

Itani O, Jike M, Watanabe N, & Kaneita Y (2016). Short sleep duration and health outcomes: a systematic review, meta-analysis, and meta-regression. Sleep Med. doi:10.1016/j.sleep.2016.08.006

Kauppila A, Pakarinen A, Kirkinen P, & Makila U (1987). The effect of season on the circulating concentrations of anterior pituitary, ovarian and adrenal cortex hormones and hormone binding proteins in the subarctic area; evidence of increased activity of the pituitary-ovarian axis in spring. Gynecological Endocrinology, 1(2), 137–150. [PubMed: 3140579]

Kecklund G, & Axelsson J (2016). Health consequences of shift work and insufficient sleep. BMJ, 355, i5210. doi:10.1136/bmj.i5210 [PubMed: 27803010]

Kloss JD, Perlis ML, Zamzow JA, Culnan EJ, & Gracia CR (2015). Sleep, sleep disturbance, and fertility in women. Sleep Medicine Reviews, 22, 78–87. doi:10.1016/j.smrv.2014.10.005 [PubMed: 25458772]

Lord C, Sekerovic Z, & Carrier J (2014). Sleep regulation and sex hormones exposure in men and women across adulthood. Pathologie Biologie, 62(5), 302–310. doi:10.1016/j.patbio.2014.07.005 [PubMed: 25218407]

Lucassen EA, Zhao X, Rother KI, Mattingly MS, Courville AB, de Jonge L, et al. (2013). Evening chronotype is associated with changes in eating behavior, more sleep apnea, and increased stress hormones in short sleeping obese individuals. PloS One, 8(3), e56519. doi:10.1371/journal.pone.0056519 [PubMed: 23483886]

Lynch KE, Mumford SL, Schliep KC, Whitcomb BW, Zarek SM, Pollack AZ, et al. (2014). Assessment of anovulation in eumenorrheic women: comparison of ovulation detection algorithms. Fertility and Sterility, 102(2), 511–518.e512. doi:10.1016/j.fertnstert.2014.04.035 [PubMed: 24875398]

Maukonen M, Kanerva N, Partonen T, Kronholm E, Konttinen H, Wennman H, et al. (2016). The associations between chronotype, a healthy diet and obesity. Chronobiology International, 33(8), 972–981. doi:10.1080/07420528.2016.1183022 [PubMed: 27246115]

Mong JA, Baker FC, Mahoney MM, Paul KN, Schwartz MD, Semba K, et al. (2011). Sleep, rhythms, and the endocrine brain: influence of sex and gonadal hormones. Journal of Neuroscience, 31(45), 16107–16116. doi:10.1523/jneurosci.4175-11.2011 [PubMed: 22072663]

Monteleone P, Mascagni G, Giannini A, Genazzani AR, & Simoncini T (2018). Symptoms of menopause - global prevalence, physiology and implications. Nature Reviews: Endocrinology, 14(4), 199–215. doi:10.1038/nrendo.2017.180

Moodie EE, & Stephens DA (2011). Marginal Structural Models: unbiased estimation for longitudinal studies. Int J Public Health, 56(1), 117–119. doi:10.1007/s00038-010-0198-4 [PubMed: 20931349]

Panda S (2016). Circadian physiology of metabolism. Science, 354(6315), 1008–1015. doi:10.1126/science.aah4967 [PubMed: 27885007]

Peuhkuri K, Sihvola N, & Korpela R (2012). Diet promotes sleep duration and quality. Nutrition Research, 32(5), 309–319. doi:10.1016/j.nutres.2012.03.009 [PubMed: 22652369]

Reiter RJ, Tamura H, Tan DX, & Xu XY (2014). Melatonin and the circadian system: contributions to successful female reproduction. Fertility and Sterility, 102(2), 321–328. doi:10.1016/j.fertnstert.2014.06.014 [PubMed: 24996495]

Reiter RJ, Tan DX, Manchester LC, Paredes SD, Mayo JC, & Sainz RM (2009). Melatonin and reproduction revisited. Biology of Reproduction, 81(3), 445–456. doi:10.1095/biolreprod.108.075655 [PubMed: 19439728]

Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. (2007). Epidemiology of the human circadian clock. Sleep Medicine Reviews, 11(6), 429–438. doi:10.1016/j.smrv.2007.07.005 [PubMed: 17936039]

Roenneberg T, Kuehnle T, Pramstaller PP, Ricken J, Havel M, Guth A, et al. (2004). A marker for the end of adolescence. Current Biology, 14(24), R1038–1039. Supplemental materials: http://

www.currentbiology.com/cgi/content/full/14/24/R1038/DC1/ doi:10.1016/j.cub.2004.11.039 [PubMed: 15620633]

Roenneberg T, & Merrow M (2007). Entrainment of the human circadian clock. Cold Spring Harbor Symposia on Quantitative Biology, 72, 293–299. doi:10.1101/sqb.2007.72.043 [PubMed: 18419286]

Rossmanith WG (1998). The impact of sleep on gonadotropin secretion. Gynecological Endocrinology, 12(6), 381–389. [PubMed: 10065163]

Sato-Mito N, Shibata S, Sasaki S, & Sato K (2011). Dietary intake is associated with human chronotype as assessed by both morningness-eveningness score and preferred midpoint of sleep in young Japanese women. International Journal of Food Sciences and Nutrition, 62(5), 525–532. doi:10.3109/09637486.2011.560563 [PubMed: 21495902]

Schernhammer ES, Rosner B, Willett WC, Laden F, Colditz GA, & Hankinson SE (2004). Epidemiology of urinary melatonin in women and its relation to other hormones and night work. Cancer Epidemiology, Biomarkers and Prevention, 13(6), 936–943.

Sharkey KM, Crawford SL, Kim S, & Joffe H (2014). Objective sleep interruption and reproductive hormone dynamics in the menstrual cycle. Sleep Medicine, 15(6), 688–693. doi:10.1016/j.sleep.2014.02.003 [PubMed: 24841109]

Shechter A, Varin F, & Boivin DB (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. Sleep, 33(5), 647–656. [PubMed: 20469807]

St-Onge MP, Mikic A, & Pietrolungo CE (2016). Effects of Diet on Sleep Quality. Advances in Nutrition, 7(5), 938–949. doi:10.3945/an.116.012336 [PubMed: 27633109]

Sun BZ, Kangarloo T, Adams JM, Sluss P, Chandler DW, Zava DT, et al. (2019). The relationship between progesterone, sleep, and LH and FSH secretory dynamics in early post-menarchal girls. Journal of Clinical Endocrinology and Metabolism. doi:10.1210/jc.2018-02400

Touzet S, Rabilloud M, Boehringer H, Barranco E, & Ecochard R (2002). Relationship between sleep and secretion of gonadotropin and ovarian hormones in women with normal cycles. Fertility and Sterility, 77(4), 738–744. [PubMed: 11937126]

Urlep Z, & Rozman D (2013). The Interplay between Circadian System, Cholesterol Synthesis, and Steroidogenesis Affects Various Aspects of Female Reproduction. Frontiers in Endocrinology, 4, 111. doi:10.3389/fendo.2013.00111 [PubMed: 24065951]

Wactawski-Wende J, Schisterman EF, Hovey KM, Howards PP, Browne RW, Hediger M, et al. (2009). BioCycle study: design of the longitudinal study of the oxidative stress and hormone variation during the menstrual cycle. Paediatric and Perinatal Epidemiology, 23(2), 171–184. doi:10.1111/j.1365-3016.2008.00985.x [PubMed: 19159403]

Watson NF, Badr MS, Belenky G, Bliwise DL, Buxton OM, Buysse D, et al. (2015). Recommended Amount of Sleep for a Healthy Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society. Sleep, 38(6), 843–844. doi:10.5665/sleep.4716 [PubMed: 26039963]

Wittmann M, Dinich J, Merrow M, & Roenneberg T (2006). Social jetlag: misalignment of biological and social time. Chronobiology International, 23(1–2), 497–509. doi:10.1080/07420520500545979 [PubMed: 16687322]

Zhu Y, Stevens RG, Hoffman AE, Tjonneland A, Vogel UB, Zheng T, et al. (2011). Epigenetic impact of long-term shiftwork: pilot evidence from circadian genes and whole-genome methylation analysis. Chronobiology International, 28(10), 852–861. doi:10.3109/07420528.2011.618896 [PubMed: 22080730]

Table 1.

Characteristics of the BioCycle Study participants (2005–2007)

	n	%
Age (years)		
Median [IQR]	24 [20–35]	
Race		
White	154	59.5
Black	51	19.7
Other	54	20.9
Body Mass Index (kg/m^2)		
Median [IQR]	23 [21–26]	
Cigarette use around the LH surge[a]		
Yes	14	5.5
Mean daily caffeine intake around the LH surge (milligrams)[a]		
Median [IQR]	43 [1–143]	
Percent of energy intake around the LH surge attributable to[a,b]:		
Alcohol		
Median [IQR][b]	0	
Fat		
Median [IQR]	34 [26–41]	
Protein		
Median [IQR]	15 [12–18]	
Carbohydrates		
Median [IQR]	50 [42–60]	
Mean daily energy intake around the LH surge (kcal)[a]		
Median [IQR]	1515 [1245–1950]	
Menses length, days[a]		
Median [IQR]	5 [4–6]	
Season around the LH surge[a]		
Winter	67	25.9
Spring	72	27.8
Summer	70	27.0
Fall	50	19.3
Average hours of sleep per day around the LH surge		
Median [IQR]	7.0 [6.3–7.8]	
Women sleeping <7 hours per day at the LH surge [a]	116	45.7
Employment and night/shift work status		
Employed, No night/shift work	103	46.0
Unemployed	44	19.6
Employed, Yes night/shift work	77	34.4

	n	%
Chronotype Scores, continuous		
Median [IQR]	4.5 [3.5–6.0]	
Categorized chronotype		
Morning (score range 2.0 to <3.5)	47	19.2
Intermediate (≤3.6 to ≤6.0)	156	63.7
Evening (>6.0 to 23.0)	42	17.1

n=number, p=p-value, IQR=interquartile range, (kg/m^2)=kilograms per meter squared, LH=luteinizing hormone, kcal=kilocalorie

The percentages in the tables reflect the proportion of women not missing information on a given characteristic. The number of women missing information: cigarette use around the LH surge, cycle 1 (n=5); daily caffeine intake around the LH surge, cycle 1 (n=7); percent of energy intake around the LH surge, cycle 1 (n=7 each for alcohol, fat, protein, and carbohydrates); daily caloric intake around the LH surge, cycle 1 (n=7); menses length in cycle 1 (n=5); night/shift work (n=35); chronotype (n=14); average daily sleep around the LH surge, cycle 1 (n=5).

[a] From the first menstrual cycle followed for each woman

[b] The IQR for percent of diet attributable to alcohol was zero.

Table 2.

Percent difference in hormone concentrations associated with daily sleep, night work, and chronotype in the BioCycle Study (2005–2007): results from linear mixed models with inverse probability weighting

	Average hours of sleep per day[a]				Night/shift work[b]				Chronotype[c]			
	Continuous, number of hours (increasing)		Dichotomous, comparing <7 hours to ≥ 7hours (reference)		Unemployed		Employed, Yes shift/night work		Morning type		Evening type	
	Percent Difference	95% CI	Percent Difference	95% CI	Percent Difference	95% CI	Percent Difference	95% CI	Percent Difference	95% CI	Percent Difference	95% CI
Testosterone	0.02	−0.7, 0.7	0.6	−1.2, 2.5	**−12.0**	**−21.4, −1.4**	**−9.9**	**−18.4, −0.4**	−0.7	−12.3, 12.5	−0.7	−12.3, 12.4
Estradiol	**3.9**	**2.0, 5.9**	**−7.0**	**−11.3, −2.5**	−6.7	−17.6, 5.7	0.4	−10.0, 12.0	−6.7	−18.8, 7.1	0.2	−12.6, 15.0
Ovulatory LH	−0.6	−4.4, 3.3	−2.4	−12.0, 8.1	7.8	−8.1, 26.4	8.9	−5.3, 25.3	1.8	−14.2, 20.8	−6.9	−21.6, 10.5
FSH	0.1	−1.2, 1.4	−3.1	−6.3, 0.2	−2.8	−11.8, 7.2	−2.4	−10.4, 6.3	4.5	−6.1, 16.4	3.5	−6.9, 15.2
Ovulatory FSH	−2.1	−4.5, 0.3	3.1	−3.1, 9.8	−1.0	−11.9, 11.2	1.4	−8.5, 12.3	−0.5	−12.3, 13.0	−1.5	−13.2, 11.9
Luteal Progesterone	**9.4**	**4.0, 15.2**	−11.3	−22.6, 1.7	9.5	−15.5, 42.0	8.9	−13.2, 36.6	−8.2	−30.8, 21.8	15.0	−13.1, 52.2

CI=confidence interval, LH=luteinizing hormone, FSH=follicle stimulating hormone. Estimates in bold are statistically significant at alpha=0.05.

These models take repeated observations into account: up to eight clinic visits per menstrual cycle and up to two menstrual cycles per woman.

[a]Average hours of sleep per day is taken from the total amount of sleep on the day of and day before each clinic visit, as reported in daily diaries. In the first model, average sleep per day is treated continuously; in the second model, those who reported <7 hours per day are compared to those reporting at least 7 hours per day (reference). Models are adjusted for: age, race, BMI, chronotype, night/shift work status, average hours of sleep per day at the prior clinic visit, hormone levels at the prior clinic visit (estradiol, progesterone, FSH, and LH), average daily caloric intake, average daily caffeine intake, cigarette use, season, and the percent of energy intake attributable to alcohol, fat, protein, and carbohydrates.

[b]Employed without shift or night work serves as the reference group for both the unemployed and the employed with shift or night work groups. Models are adjusted for age, race, BMI, chronotype, season, and hormone levels at the prior clinic visit (estradiol, progesterone, FSH, and LH).

[c]Intermediate chronotype serves as the reference for both morning and evening chronotypes. Models are adjusted for age, race, BMI, night/shift work status, season, and hormone levels at the prior clinic visit (estradiol, progesterone, FSH, and LH).

Table 3.

Risk of sporadic anovulation associated with sleep characteristics in the BioCycle Study (2005–2007)

	Risk of Anovulation	
	RR	**95% CI**
Average daily sleep duration (in hours) at the LH surge [a]		
Continuous number of hours (increasing)	0.79	0.59, 1.06
<7 hours compared to ≥7 hours	1.63	0.84, 3.16
Employment and night/shift work status [b]		
Employed, No shift/night work	reference	
Unemployed	0.65	0.24, 1.73
Employed, Yes shift/night work	0.70	0.32, 1.56
Chronotype [b]		
Morning	2.50	0.93, 6.77
Intermediate	reference	
Evening	1.20	0.50, 2.86

RR=risk ratio, CI=confidence interval.

These modified Poisson regression models take repeated observations into account: up to two menstrual cycles per woman.

[a] Average hours of sleep per day is taken from the total amount of sleep on the day of and day before the clinic visit around the LH surge, as reported in daily diaries. In the first model, average sleep per day is treated continuously; in the second model, those who reported <7 hours per day are compared to those reporting at least 7 hours per day (reference). Models are adjusted for: age, race, BMI, chronotype, night/shift work status, season; and at the time of the LH surge in each cycle, average daily caloric intake, average daily caffeine intake, cigarette use, and percent of energy intake attributable to alcohol, fat, protein, and carbohydrates.

[b] Adjusted for age, race, body mass index (BMI), chronotype (night/shift work model), night/shift work status (chronotype model), and season.