Anti-Müllerian hormone levels in nurses working night shifts

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Abstract

Our objective was to examine associations between night shift work and serum anti-Müllerian hormone (AMH) levels. We analyzed 1,537 blood samples from premenopausal female nurses in the Nurses’ Health Study II, assayed for AMH. Rotating or permanent night shifts worked in the two weeks before blood collection and years of rotating night shift work were obtained via questionnaire. We found no associations between recent night shifts or rotating night shift work and AMH. The median difference in AMH was 0.3 (95% CI: −0.4, 0.8) ng/mL for ≥5 versus 0 recent night shifts and −0.1 (95% CI: −0.4, 0.3) ng/mL for ≥6 versus 0 years of rotating night shift work. Although we found no associations between night shift work and AMH, this does not preclude associations between night shift work and fertility operating through other mechanisms.

INTRODUCTION

Shift work involving circadian disruption, such as night shift work and rotating night shift work, is associated with increased risks for cancer, cardiovascular disease, and...
gastrointestinal disorders. A growing body of literature has examined the effects of circadian-disruptive shift work on reproductive outcomes such as fertility and menstrual function; these outcomes are dependent on hormones whose cyclic rhythms might be disrupted by night shift work.

Many of these studies have investigated time to pregnancy, the number of months of unprotected intercourse before a couple achieves pregnancy. Some have found that women who perform shift work take longer to become pregnant, while others have found no association. Although time to pregnancy is easily measured in questionnaires, it cannot distinguish between male and female fertility problems and it is difficult to measure among couples who do not eventually conceive or who have unplanned pregnancies.

The search for a better measure of female reproductive potential led to anti-Müllerian hormone (AMH). AMH is a hormone produced by the cells of the ovarian follicles, the structures in the ovary that encircle the egg and prime it for ovulation. Serum AMH levels mirror women’s fertility over time: increasing in childhood, peaking in early adulthood, and declining to undetectable levels at menopause. They are considered a valid measure of ovarian reserve, the number of eggs remaining in the ovary. AMH levels are correlated with other validated measures of ovarian reserve, such as counting the number of follicles by ultrasound. Although AMH and other measures of ovarian reserve are not strongly associated with time to pregnancy, they are associated with time to menopause (length of the reproductive lifespan). Little research has been conducted on the effects of night shift work on time to menopause. But, associations between night shift work and adverse pregnancy outcomes, menstrual function, and fecundability suggest that time to menopause could be another reproductive outcome affected by night shift work.

There are two previous studies of shift work and ovarian reserve; only one specifically investigated night shift work. This study found that night shift work was associated with diminished ovarian reserve (shorter time to menopause) when using one measure (oocyte yield), but was not associated with another measure (antral follicle count). The other study found that shift work of any kind (not night shift work specifically) was not associated with AMH levels or antral follicle count.

The objective of this study was to contribute further evidence for or against an association between night shift work and ovarian reserve. To do this, we examined serum AMH levels in a large prospective cohort study of female nurses working night shifts. Because other studies have found associations between night shift work and adverse reproductive outcomes, we hypothesized that nurses working night shifts would have lower AMH levels than nurses not working night shifts.

**METHODS**

The Nurses’ Health Study II (NHSII) is a longitudinal study of 116,429 female registered nurses in the United States who enrolled between the ages of 25 to 42 years. Beginning in...
1989, nurses completed mailed questionnaires every two years, which collected information on health conditions and health-related behaviors.

Our study population was drawn from 29,611 NHSII participants aged 32 to 54 years with no history of cancer who participated in an NHSII biomarker substudy between 1996 and 2000. All women gave written informed consent before participating and the study was approved by the institutional review board of Brigham and Women’s Hospital. This research was completed in accordance with the Helsinki Declaration.

Biomarker substudy participants were split into two groups: one provided blood samples timed to the follicular and luteal phases of their menstrual cycle (“timed” samples, n = 18,521) and one provided a single blood sample at an arbitrary time (“untimed” samples, n = 11,090). Nurses providing timed samples were premenopausal, not currently using hormonal contraceptives or hormonal medications, and were not pregnant or breastfeeding within the past 6 months. Nurses providing untimed samples were either ineligible for the timed samples or declined to provide timed samples.

At the time of blood collection, participants completed a short questionnaire that included questions about sociodemographics, lifestyle, and recent night shift work. Blood samples were shipped on ice to the study laboratory, processed, and stored in the vapor phase of liquid nitrogen.

**Participant selection**

Serum AMH levels were assayed for three studies conducted among NHSII biomarker substudy participants between 2014 and 2018: a case-control study of breast cancer, a case-control study of early menopause, and a cross-sectional study. Only control women from the case-control studies were eligible for our analysis; all participants from the cross-sectional study were eligible. For the 19 women in more than one study, we randomly selected one AMH value. Our analytic sample included the 389 breast cancer controls, 410 early menopause controls, and 789 women from the cross-sectional study. All participants were premenopausal at time of blood collection.

**AMH measurement**

AMH was assayed using the Ansh Labs picoAMH assay (Webster, TX) with a limit of detection (LOD) of 0.01 pg/mL. All samples with values below the LOD were assigned a value of LOD/2. Coefficients of variation were 9% for the case-control studies and 17% for the cross-sectional study.

Because blood samples from each study were assayed in separate batches, we applied a batch correction algorithm created for NHSII to account for potential differences between the three assays. The median AMH levels were 2.4 and 1.8 ng/mL before and after correction and the Pearson correlation coefficient between the two versions was 0.74.

**Night shift work**

We used two measures of night shift work: recent night shift work and years of rotating night shift work.
Recent night shift work.—On the questionnaire at time of blood collection, nurses were asked, “How many night shifts did you work in the past 2 weeks?” with response options of 0, 1–2, 3–4, 5–6, or >6 nights. We created three categories with adequate sample size for analysis: no recent nights (0), 1–4 recent nights, and ≥5 recent nights.

Years of rotating night shift work.—At baseline in 1989, participants reported how many years of rotating night shifts they had worked in their career, with response options of 0, 1–2, 3–5, 6–9, 10–14, 15–19, and ≥20 years. Rotating night shift work was defined as working at least 3 nights per month in addition to other days and evenings that month. In the 1991, 1993, and 1997 biennial questionnaires, nurses updated their duration of rotating night shift work over the prior two years (response options: 0, 1–4, 5–9, 10–14, 15–19, ≥20 months). For any questionnaire with missing data on rotating night shift work (including the 1995 and 1999 questionnaires, which did not include these questions), we carried forward the response from the prior questionnaire once. We added the years of rotating night shift work at baseline to the months of rotating night shift work reported through the time of blood collection to estimate the number of years each nurse worked rotating night shifts. We used category midpoints for the calculation, except for the highest categories, for which the category minimum was used. For the analysis, we created three groups with adequate sample size for analysis: 0, 1–5, and ≥6 years.

Covariates

Based on prior studies, we identified potential confounders age, race/ethnicity, body mass index (BMI), current smoking status, and recent hormonal contraceptive or hormonal medication use (“hormone use”, for brevity). Age, BMI (kg/m²), and current smoking status were collected on the questionnaire at time of blood collection; race/ethnicity (non-Hispanic white, other) was collected on the NHSII biennial questionnaires. Women smoking ≥1 cigarettes per day were categorized as current smokers and women smoking 0 cigarettes per day were considered nonsmokers. Hormone use in the past 6 months was asked on the questionnaire at time of blood collection for women in the timed sample group, but not for women in the untimed sample group. For these women, we obtained information on hormonal contraceptive use from the biennial questionnaire preceding blood collection (between 0 to 2 years prior to blood collection for most women). We additionally included age² and BMI² in the models because these terms were predictors of AMH levels (p<0.05).

Statistical analyses

We performed quantile regression at the 10th, 25th, 50th, 75th, and 90th percentiles of the AMH distribution and estimated differences in AMH levels and their 95% confidence intervals (CI) to describe relationships between night shift work and AMH levels. Quantile regression results are interpreted similarly to those from linear regression. In linear regression, they represent differences in the mean of the outcome between the exposed and unexposed, whereas in quantile regression they represent differences in the quantile value of the outcome.³⁰ For example, quantile regression at the 50th percentile corresponds to the difference in median AMH levels between the exposed and unexposed groups.
Once we examined the data, there was too little variability in hormone use and race/ethnicity to include these as covariates in the model. \(^{31}\) We controlled for potential confounding by hormone use by excluding women who reported hormone use from the primary analysis and controlled for race/ethnicity in a sensitivity analysis (described below).

We adjusted all models for age, age\(^2\), BMI, BMI\(^2\), and smoking status and used SAS 9.4 (Cary, NC) for the analyses.

**Exclusions**

Of the AMH samples from 1,588 individual women, we excluded 23 with missing BMI, 6 with BMI outliers (<15 or >80 kg/m\(^2\)), 1 with missing smoking status, 19 with recent hormone use, and 2 with missing data on recent hormone use. From the remaining 1,537 women, we excluded those with missing data on recent night shift work (n = 8) and years of rotating night shift work (n = 57) for each shift work analysis, respectively.

**Sensitivity analyses**

We conducted six sensitivity analyses, as follows. First, we restricted the analysis to non-Hispanic white women to address potential confounding by race/ethnicity. This left 1,486 participants in the analysis. Second, we restricted the analysis to women providing timed luteal phase blood samples to account for differences in AMH levels by phase of the menstrual cycle, leaving a sample size of 1,415 women. \(^{32}\) Third, we wanted to exclude women with polycystic ovary syndrome (PCOS), who typically have high AMH levels. Because we had no reliable method of identifying women with PCOS in our study population and because there is no accepted AMH cutoff for PCOS, we excluded women with AMH levels >4.7 ng/mL (88\(^{th}\) percentile of the AMH distribution), a cutpoint proposed for PCOS screening in a meta-analysis. After this exclusion, the sample size was 1,353. \(^{33}\) Fourth, we excluded all participants from the early menopause case-control study, leaving 1,145 participants. Because AMH levels are associated with age at menopause, including only the control women results in exclusion of women with the lowest AMH levels in the population. \(^{23}\) Fifth, we stratified the analysis by age (<40, ≥40 years) to determine if the association differed as women approached menopause (sample sizes: 840 for age <40 years, 689 for age ≥40 years). Sixth, because our analysis excluded current hormonal contraceptive users, our study population might have preferentially included women with known infertility. We stratified by pregnancy history (0, ≥1) to investigate potential effects of this selection (sample sizes: 179 for no prior pregnancy, 1,350 for ≥1 pregnancies).

**RESULTS**

The median AMH level was 1.8 ng/mL, with an interquartile range of 0.9 to 3.2 ng/mL. Characteristics of study participants, age-standardized and stratified by night shift work status, are presented in Table 1. Nurses with and without night shift work were similar on most characteristics.

13\% of participants (206/1,529) worked one or more night shifts in the two weeks before blood collection (Table 2). This included 130 women (9\%) who worked 1–4 recent night
shifts and 76 (5%) who worked 5 or more. 70% of nurses (1,033/1,480) worked rotating night shifts at some point in their career, most for less than 6 years.

In adjusted quantile regression models, we found no associations between recent night shift work or years of rotating night shift work and AMH levels at any percentile of the AMH distribution (Table 2).

Interpretation of results was mostly unchanged in sensitivity analyses restricting to non-Hispanic white women, timed luteal samples, and AMH values ≤4.7 ng/mL and when excluding women from the early menopause case-control study, stratifying by age, and stratifying by pregnancy history (Figure 1). The greatest differences were suggestive associations between years of rotating night shift work and median AMH levels after excluding women from the early menopause case-control study (−0.3, 95% CI: −0.6, 0.0 for 1–5 versus 0 years) and among women with no pregnancies prior to blood collection (−0.7, 95% CI: −1.1, 0.0 for ≥6 versus 0 years).

**DISCUSSION**

In a cohort of female nurses, we found no association between recent night shift work or years of rotating night shift work and AMH levels.

In two sensitivity analyses, we found suggestive associations between years of rotating night shift work and median AMH levels. However, the confidence intervals were wide and we did not see dose-response associations with number of years of rotating night shift work. These associations might have been due to chance.

There are two other studies of shift work and ovarian reserve. One found no difference in mean AMH level or antral follicle count (a measure of ovarian reserve correlated with AMH) between shift workers and non-shift workers. These results are difficult to compare to ours because no definition of shift work was provided and the analyses were not adjusted for age, an important confounder. The other study found an association between shift work (night, evening, or rotating shifts) and lower oocyte yield, another marker of ovarian reserve, among patients at a fertility clinic. However, this same study found no association between shift work and antral follicle count, providing no clear conclusion about associations between shift work and ovarian reserve.

Absence of an association between night shift work and AMH (or other measures of ovarian reserve) does not rule out an association between night shift work and fertility. Recent studies show that AMH is more correlated with shorter time to menopause and early menopause than it is with fecundability. If night shift work acts on fertility through a mechanism not involving ovarian reserve or AMH secretion, we would not expect an association with AMH. In NHSII and other study populations, rotating night shift work is associated with menstrual cycle disturbances, including short, long, and irregular cycles, all of which could affect fertility. Night shift work can also affect levels of reproductive hormones with circadian patterns (e.g., follicle-stimulating hormone and luteinizing hormone), but whether or not it affects AMH in this way is unknown. There is some
evidence that AMH levels have circadian patterns, with the lowest levels in the early morning (4:00–6:00 am).37

We used a single measurement of AMH levels to characterize ovarian reserve. AMH levels are fairly stable over the menstrual cycle, with any variability not large enough to warrant timing samples to specific phases of the menstrual cycle.32 Accordingly, in our sensitivity analysis restricted to luteal phase samples, we found similar results to when all samples were included.

We attempted to exclude nurses with PCOS in a sensitivity analysis by excluding women with AMH levels >4.7 ng/mL, a cutpoint derived from a meta-analysis.33 This strategy had limitations, including that AMH levels are strongly correlated with age, and so a single cutpoint would not identify PCOS cases equally well in every age group. However, if the association between shift work and AMH levels is the same in women with PCOS as in other women, exclusion of women with PCOS would be unnecessary. We had no information to know if this was true. The results from this sensitivity analysis were no different from the main analysis.

Ideally, our analysis of recent night shift work would have separated nurses working permanent night shifts from those working rotating night shifts, the latter of which can worsen circadian disruption. However, we did not know the work schedules of nurses working recent night shifts. We suspect that those working ≥5 night shifts in the prior two weeks were more likely to be permanent (non-rotating) night shift workers, but we have no information available to confirm this suspicion.

We also had few details about nurses’ rotating night shifts, such as frequency of night shifts and direction of rotation, both of which could affect the severity of circadian disruption.38 Misclassification of years of rotating night shift work was also possible. Information on rotating night shift work was not collected on the 1995 and 1999 questionnaires; our assumption that nurses continued their previous schedules could have either overestimated or underestimated their duration of rotating night shift work. Our variable for years of rotating night shift work did not take into account how recently nurses were working rotating night shifts. If rotating night shift work does not have a long-term effect on AMH levels and if most nurses were not recently working rotating night shifts, we would expect to see no association between these variables.

Residual confounding is possible, both by measured and unmeasured confounders. We controlled for age, a known and important confounder because young age is strongly associated with both working night shifts and higher AMH levels.39, 40 Confounding by unmeasured or overlooked confounders remains a concern because AMH was only recently identified as a biomarker for ovarian reserve, and there is still conflicting information about what factors affect AMH levels.39 We controlled for BMI, smoking, hormone use, and race/ethnicity, variables associated with AMH levels in prior studies and that could differ by night shift work status.

Our study had several strengths. We had measures of both short-term (recent night shift work) and long-term (years of rotating night shift work) exposure, allowing us to investigate
either acute or chronic effects of night shift work. The sample size was large enough to use multi-level, instead of dichotomous, exposures.

Quantile regression also provided several advantages over linear regression. The AMH distribution was not normally distributed, which made the 50th percentile (median) a more natural measure of central tendency to model than the mean. By conducting regressions at multiple quantiles of the AMH distribution, we were also able to investigate differences in the association over the distribution, although we found little difference. Quantile regression is less sensitive to outliers and therefore might be more robust to potential inclusion of women with high AMH values due to PCOS. Our results were also unaffected by coding values below the LOD as LOD/2 instead of other options such as LOD/√2, because these decisions do not affect the percentiles of the distribution like they would affect the mean in linear regression.

We found no overall association between night shift work and serum AMH levels, a marker of ovarian reserve. Given the mixed results of studies of night shift work and fecundability and the association between night shift work and menstrual cycle disruption, an association between night shift work and female fertility that does not operate through ovarian reserve might exist.

ACKNOWLEDGEMENTS

The findings and conclusions in this report are those of the authors and do not necessarily reflect the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. The authors wish to thank the Nurses’ Health Study II participants for their generous time and dedication to the study and Eileen Lividoti Hibert for invaluable assistance and support in the development of this manuscript.

FUNDING

Funding for this work was provided by grants OH009803, R01CA67262, R01CA178949, R01HD078517, and UM1CA17626 from the National Institutes of Health and by contract number 200–2015-M-61780 to the Brigham and Women’s Hospital from the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health.

REFERENCES


Figure 1.
Adjusted associations between night shift work and median AMH levels (ng/mL) with 95% confidence intervals. Upper panel, by night shifts worked in the two weeks before blood collection. Lower panel, by years of rotating night shift work. Analyses: original; restricting to non-Hispanic white nurses; restricting to timed samples; excluding AMH levels >4.7 ng/mL; excluding participants from the early menopause study; stratifying by age; and stratifying by pregnancy history.
Table 1.
Age and age-standardized participant characteristics at time of blood collection by night shift work — Nurses’ Health Study II, 1996–2000.

<table>
<thead>
<tr>
<th></th>
<th>Recent Night Shift Work&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ever Worked Rotating Night Shifts&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (N = 206)</td>
<td>No (N = 1,323)</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>39.9 (3.6)</td>
<td>39.4 (3.5)</td>
</tr>
<tr>
<td>Body mass index, kg/m², mean (SD)</td>
<td>24.6 (5.1)</td>
<td>24.6 (3.8)</td>
</tr>
<tr>
<td>Race/ethnicity, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>All others</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current non-smoker</td>
<td>97</td>
<td>94</td>
</tr>
<tr>
<td>Current smoker</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Worked at least one night shift in the two weeks before blood collection.

<sup>b</sup>At least one month of rotating night shift work during the nurse’s career.
Table 2.

Adjusted differences in serum AMH levels between night and non-night shift workers at five percentiles of the AMH distribution — Nurses’ Health Study II, 1996–2000.

<table>
<thead>
<tr>
<th>Night shifts worked in the two weeks before blood collection</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 night shifts</td>
<td>1,323</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1–4 night shifts</td>
<td>130</td>
<td>0.0</td>
<td>(−0.2, 0.3)</td>
<td>0.1</td>
<td>(−0.2, 0.5)</td>
</tr>
<tr>
<td>≥5 night shifts</td>
<td>76</td>
<td>0.0</td>
<td>(−0.2, 0.4)</td>
<td>0.1</td>
<td>(−0.2, 0.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Years of rotating night shift work</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 years</td>
<td>447</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1–5 years</td>
<td>785</td>
<td>0.0</td>
<td>(−0.2, 0.1)</td>
<td>−0.1</td>
<td>(−0.3, 0.1)</td>
</tr>
</tbody>
</table>

\[\text{Adjusted for age}, \text{age}^2, \text{body mass index}, \text{body mass index}^2, \text{and current smoking status.}\]