Association between circadian rhythm disruption and polycystic ovary syndrome

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Objective: To explore the association of circadian rhythm disruption with polycystic ovary syndrome (PCOS) and the potential underlying mechanism in ovarian granulosa cells (GCs).

Design: Multicenter questionnaire-based survey, in vivo and ex vivo studies.

Setting: Twelve hospitals in China, animal research center, and research laboratory of a woman’s hospital.

Patients/Animals: A total of 436 PCOS case subjects and 715 control subjects were recruited for the survey. In vivo and ex vivo studies were conducted in PCOS-model rats and on ovarian GCs collected from women with PCOS and control subjects.

Intervention(s): The PCOS rat model was established with the use of testosterone propionate.

Main Outcome Measure(s): Assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq), RNA sequencing, rhythmicity analysis, functional enrichment analysis.

Result(s): There was a significant correlation between night shift work and PCOS. PCOS-model rats presented distinct differences in the circadian variation of corticotropin-releasing hormone, adrenocorticotropic hormone, prolactin, and a 4-h phase delay in thyrotropic hormone levels. The motif enrichment analysis of ATAC-seq revealed the absence of clock-related transcription factors in specific peaks of PCOS group, and RNA sequencing ex vivo at various time points over 24 hours demonstrated the differential rhythmic expression patterns of women with PCOS. Kyoto Encyclopedia of Genes and Genomes analysis further highlighted metabolic dysfunction, including both carbohydrate and amino acid metabolism and the tricarboxylic acid cycle.

Conclusion(s): There is a significant association of night shift work with PCOS, and genome-wide chronodisruption exists in ovarian GCs. (Fertil Steril 2020; : - . © 2020 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, night shift work, circadian rhythm, ovarian granulosa cell

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Poly cystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in women of reproductive age, characterized by hyperandrogenism and abnormal follicular development (1). Aside from being the leading cause of absent or infrequent menstruation and anovulatory infertility (1), PCOS is associated with an increased risk of obesity, type 2 diabetes, cardiovascular disease, and certain cancers (2, 3). The circadian rhythm, which coordinates with the Earth’s rotation, orchestrates the temporal organization of diverse physiologic events, such as endocrinology and metabolism, involving both central nervous system and peripheral tissues (4). As a typical example, the light exposure and the alteration of sleep and food patterns during shift work lead to an acute circadian misalignment (5). Therefore, it is worth exploring the potential link among circadian disruption, night shift work, and PCOS.

In central circadian timing system, a master pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) orchestrates circadian outputs through a rhythmic secretion pattern of regulatory hormones such as melatonin and corticotropin–releasing hormone (CRH)/adrenocorticotropic hormone (ACTH) (6–8). On one hand, pineal melatonin synthesis is regulated by the central circadian clock within the hypothalamic SCN, which is also directly inhibited by light (9). Melatonin rhythms are disrupted in both permanent night shift workers and women with PCOS (10, 11). The levels of melatonin, together with its metabolite, increase in the serum (12, 13) and urine (14) of women with PCOS. On the other hand, the excitation of SCN central pacemaker neurons controls ACTH secretion from the pituitary corticotropes via the release of CRH (15). Nonetheless, evidence for the exact release pattern of CRH/ACTH in women with PCOS remains limited (16, 17). It is striking that not only the SCN but also the periphery, even cultured cells in vitro, retain their self-sustained circadian rhythmicity, profoundly affecting diverse physiologic processes (18, 19).

Physiologically, the processes of follicular development in mammalian ovaries display robust circadian rhythms, which are coordinated with light cycles and endocrine signals (20). The rhythmic expression of the core clock genes, such as CLOCK and BMAL1, and clock-controlled genes, including steroidogenic acute regulatory protein (STAR) (21) and peroxisome proliferator–activated receptor gamma coactivator 1-alpha (PPARGC1A) (22), are precisely regulated in the ovary, which is required for ovary-specific rhythms (23). Based on the available evidence, we hypothesized that there may exist an association between circadian rhythm disruption and PCOS. In this study, we investigated the correlation of night shift work with PCOS and the potential underlying mechanism in a series of population, in vivo, and ex vivo studies.

**MATERIAL AND METHODS**

**Multicenter Questionnaire-Based Survey**

This survey was conducted from June 2016 to May 2017 in 12 hospitals in China (see Supplemental Materials, available online at www.fertstert.org). In this study, only women aged 21–45 years without any sleep disorders caused by neurologic, psychiatric, respiratory, or other disorders were recruited. All of the women came to the hospitals for receiving treatment of reproductive disorders (such as irregular menstruation or infertility) or for a health screening (control). Patients were diagnosed with PCOS according to the Rotterdam Consensus (European Society of Human Reproduction and Embryology [ESHRE]/American Society for Reproductive Medicine [ASRM] criteria) (2) by obstetrician-gynecologists. The exclusion criteria were pregnancy, endocrine- and metabolism-related disorders, ovarian cysts or tumors, and uterine disorders. All of the women were free from medication known to affect metabolic or reproductive functions within the 3 months preceding their enrollment. Written informed consent was obtained from every participant. Data were collected using a structured questionnaire. Information about age, height, weight, sleeping, tobacco consumption, and alcohol intake status was collected. Participants were asked whether they did night shift work and about the shift types, such as permanent or rotating night work. Night shift work was defined as a working schedule that involved partly or entirely working between midnight and 6:00 AM (24). Only the patients’ work history before their PCOS diagnosis was considered and collected. Because of missing information in the questionnaire, 17 women with PCOS and 29 control women were excluded from the analyses. The present analyses include data from 436 PCOS patients and 715 control subjects.

**Establishment of the PCOS Rat Model**

Twenty neonatal female Sprague-Dawley rats were provided by the Laboratory Animal Research Center, Zhejiang Chinese Medical University, Hangzhou, China. They were randomly divided into the PCOS-model and control groups (n = 10 in each group). On the 9th day after birth, the PCOS rat model was induced by subcutaneous injection of testosterone propionate (Solabio) at the dose of 0.1 mg/0.004 mL olive oil per g of animal (25); the control group received olive oil only. Following protocol, the PCOS rat model was validated (26, 27). More detailed information is provided in the Supplemental Materials. The study was performed according to the Care and Use of Laboratory Animals protocol of the National Research Council of China and was approved by the Zhejiang University Ethics Committee.

**Femoral Artery Catheterization and Blood Sampling**

Catheters were placed in the femoral artery of each rat and connected to a compact perfusor for continuous constant infusion of 0.1% heparinized saline (28). Blood samples were collected every 4 hours during the following 24 hours on the day after surgery, beginning from 10:00 pm. These samples were then centrifuged for 10 minutes at 1,000g. Serum was separated and stored at −80°C for further estimation.
Serum Biochemical Assays

The Milliplex Map Rat Pituitary Magnetic Bead Panel—Endocrine Multiplex Assay was used to detect the serum levels of LH, FSH, thyrotropic hormone (TSH), and prolactin (PRL) (Merck Millipore). Serum levels of ACTH, CRH, SHBG, and antimüllerian hormone (AMH) were measured with the use of commercially available ELISA kits (Cusabio). The concentrations of serum total testosterone (TT) was measured with the use of another ELISA kit (Sigma-Aldrich). Free androgen index (FAI) was calculated as TT (nmol/L) divided by SHBG (nmol/L) × 100 (29). Melatonin was assayed with the use of ELISA kits from Abcam according to the manufacturer’s instructions.

Patient Sample Collection

Among the subjects included in the survey, 36 women with PCOS and 36 healthy control women were recruited for the sample collection at the Women’s Hospital, School of Medicine, Zhejiang University. Peripheral blood samples were collected between 8:00 AM and 10:00 AM on day 2 of the menstrual cycle and centrifuged for 10 minutes at 1,000 g. The serum was separated and stored at −80°C for melatonin estimation.

The primary human GCs for ex vivo study were collected from three infertile women with PCOS and three infertile women with tubal blockage as control subjects, who were referred to the Women’s Hospital, School of Medicine, Zhejiang University, undergoing IVF-ET. PCOS was diagnosed according to the Rotterdam Consensus (ESHRE/ASRM criteria) (2). The exclusion criteria were pregnancy, endocrine- and metabolism-related disorders, ovarian cysts or tumors, and uterine disorders according to laboratory, ultrasound, or laparoscopy examinations. The control women had regular menstrual cycles and normal sex hormone levels. The long agonist protocol for controlled ovarian hyperstimulation was used. Women with sleep disorders or a history of night work in the past 2 years were excluded. The characteristics of the included patients are presented in Supplemental Table 2 (available online at www.fertstert.org). The GCs were obtained by follicular aspiration from the women undergoing oocyte retrieval for IVF-ET at the time point of 1:00 PM going oocyte retrieval for IVF-ET at the time point of 1:00 PM were obtained by follicular aspiration from the women under-}

RNA Sequencing

Total RNA was extracted from GCs with the use of Trizol reagent (Invitrogen) according to the manufacturer’s instructions. Then the paired-end sequencing was performed on the Illumina X10 platform (LC Sciences) according to the vendor’s recommended procedure. More detailed information is presented in the Supplemental Materials.

Rhythmicity Analysis

For the detection of circadian rhythmicity, the Rhythmicity Analysis Incorporating Nonparametric (RAIN) method was used on all samples from each time point. A P value of <.0001 was considered to be strongly significant. The heatmaps were achieved by means of the median normalization method and generated by MultiExperiment Viewer v4.9.

Functional Enrichment Analysis

Metascape analysis was performed with the use of the significantly rhythmic genes for functional enrichment analysis (33). Protein-protein interaction (PPI) enrichment analysis was carried out by means of Metascape and Cytoscape (v 3.6.1) with the use of the BioGrid (34), IntWeb.IM (35), and OmniPath (36) databases. For each cluster, the hypergeometric test was applied to obtain enriched Gene Ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways at the set significance level (P <.05).

Statistical Analyses

All analyses were conducted with the use of SPSS version 20.0 (IBM Corp.). Data are expressed as mean ± SEM. Unpaired Student t tests and one-way analysis of variance with post hoc tests or their equivalent nonparametric tests were used to compare numeric data. Chi-square tests were used to compare categoric data. Multivariable unconditional logistic regression models were used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between night shift work and PCOS. Multivariable models were adjusted for age, body mass index (BMI), tobacco consumption, alcohol intake, occupation, and family history of PCOS. All tests of statistical significance were two sided with a P value of <.05.
RESULTS

Night Shift Work and PCOS Risk

The descriptive characteristics of the PCOS cases and controls are presented in Table 1. A significantly positive family history of PCOS was found among women with PCOS compared with the control women. No significant differences were found between the PCOS and control groups regarding age, BMI, occupation, tobacco consumption, and alcohol intake. After adjusting for potential confounders, there existed a significant correlation between night shift work and PCOS (OR 1.54, 95% CI 1.18–2.75; Supplemental Table 1, available online at www.fertstert.org). This association was also observed among those with rotating, but not permanent, night shifts (rotating vs. day work: OR 2.00, 95% CI =1.16–3.44; permanent vs. day work: OR 1.54, 95% CI 0.80–2.95). A significant association existed between night shift work for a longer time (>2 years) with PCOS (OR 2.08, 95% CI 1.15–3.73).

Roles of Melatonin in the Link Between Night Shift Work and PCOS

Melatonin levels in the serum collected at 9:00 AM in PCOS patients varied from 14.20 to 27.30 pg/mL, whereas they ranged from 10.03 to 29.02 pg/mL in the control subjects. We did not find any significant difference between women with PCOS and control women (Fig. 1A). We then divided each group into two subgroups based on whether the subjects had worked night shifts in the past 2 years. No statistically significant differences in the serum melatonin levels were observed among these four subgroups, as shown in Figure 1B.

Circadian Variations of Peripheral Hormone Levels in PCOS Rats

Out of ten rats, the PCOS model was successfully established in 8 rats, as indicated by irregular estrous cycle, elevated serum FAI levels (Fig. 1C), and multiple cystic follicles and decreased numbers of GCs as well as corpora lutea observed with the use of hematoxylin and eosin staining (Fig. 1D). To explore the circadian variations of hormones in peripheral blood of PCOS rats, a wide panel of hormones was measured. The melatonin levels over all the time points in PCOS rats were higher, but not significantly, than in the control rats (Fig. 1E). Compared with control rats, the CRH levels in PCOS rats were significantly higher at 6:00 AM (P=.001) and significantly lower at 10:00 AM (P=.031) (Fig. 1F). The analysis of ACTH levels revealed that the greatest difference between PCOS rats and controls occurred at 6:00 AM (P=.015), when both ACTH and CRH levels peaked in PCOS rats, but reached a nadir in control rats (Fig. 1G). For TSH levels, PCOS rats showed a 4-hour phase delay from the control rats, without any significant differences at all time points (Fig. 1H). The levels of PRL in PCOS rats were significantly higher than in control rats at 22:00 PM (P=.016; Fig. 1I). In PCOS rats, both the ratios of LH to FSH and AMH levels were similar to the control rats (Figs. 1J and 1K).

Landscapes of Transcription and Chromatin Accessibility in GCs of Women with PCOS and Control Women

To explore the transcriptional regulation in human GCs of the PCOS and control groups, we assessed the transcriptional status and chromatin accessibility by means of RNA-seq and ATAC-seq (n = 3 in each group). Global analysis of transcriptomic data revealed a dramatically changed pattern of gene expression (Supplemental Fig. 1A, available online at www.fertstert.org) in GCs of PCOS patients. Circos plot was used to visualize global chromatin accessibility among all samples (Supplemental Fig. 2, available online at www.fertstert.org). The specific and common peaks in each group were visualized with heatmaps (Supplemental Fig. 1B). Here, 3,939 (27.5%) ATAC peaks were common peaks between PCOS and control group, while 5,792 (40.5%) peaks and 4,576 (32.0%) peaks were specific peaks of the control group and the PCOS group, respectively.

Motif Enrichment Analysis and Transcription Factor–Binding Site Prediction in Human GCs

HOMER was used to identify enriched putative transcription factor (TF) known motifs in the specific peaks of open chromatin regions between the PCOS and control groups. Excluded from the common motifs of both groups, the top 12 most significantly enriched motifs are shown in Supplemental Figure 1C. The clock-related TFs including

<table>
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<th>PCOS (n = 436)</th>
<th>P value</th>
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<tr>
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<td>281</td>
<td>64.45</td>
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<tr>
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<td>132</td>
<td>30.28</td>
</tr>
<tr>
<td>≥28</td>
<td>32</td>
<td>23</td>
<td>5.28</td>
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<td>82.80</td>
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<tr>
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<td>75</td>
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<td>6°</td>
<td>167</td>
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</table>

Note: PCOS = polycystic ovary syndrome.

(a) Categories according to the National Occupational Classification Code of the People’s Republic of China (2015): 1) the principal state ministries, party group organizations, enterprises, and institutions; 2) professional technologists; 3) affair management and related jobs; 4) business services; 5) agriculture, forestry, stock breeding, fisheries, and irrigation; 6) production, transportation, equipment operation, and related.

**Wang. Circadian rhythm disruption and PCOS. Fertil Steril 2020.**
Circadian variation of peripheral hormone levels in PCOS and control subjects. (A) The serum melatonin levels of women with PCOS and controls (n = 36 in each group). (B) Detailed subgroups analysis (n = 18 in each group). (C) Serum free androgen index (FAI) of PCOS (n = 8) and control (n = 7) rats. (D) Rat ovarian histological examination using HE staining, with ×5 magnification. (E–K) Circadian variation of peripheral hormone levels in PCOS and control rats (n = 5 for each group): (E) melatonin, (F) corticotropin-releasing hormone (CRH), (G) adrenocorticotropic hormone (ACTH), (H) thyrotrophic hormone (TSH), (I) prolactin (PRL), (J) LH/FSH, (K) antimüllerian hormone (AMH). Data presented as mean ± SEM. Unpaired two-tailed Student t test, one-way analysis of variance, and the Bonferroni post test were performed for the data. *P < .05 was considered to be statistically significant. PCOS = polycystic ovary syndrome.

Altered transcriptome profiles in primarily cultured granulosa cells (GCs) of women with polycystic ovary syndrome (PCOS). (A–E) Volcano plots of differential gene expression at various time points, with fold change as the abscissa and $-\log_{10}(P \text{ value})$ as the ordinate. Red and blue splashes represent genes that were significantly up- or down-regulated in PCOS, respectively. Gray splashes represent genes without significantly different expression. (F) The corresponding total numbers of notably up- and down-regulated genes at various time points. (G) Transcriptional expression of core clock genes in primarily cultured GCs of women with PCOS (orange) and control women (blue). Transcripts that were identified as circadian are represented by fitted regression curves. $P<.05$.

Circadian transcriptional rhythms of polycystic ovary syndrome (PCOS) and control groups. (A) Venn diagram showing the genes with a strong circadian rhythm in expression detected by the Rhythmicity Analysis Incorporating Nonparametric method. (B) Median-normalized, acrophase-ordered expression heatmaps of cycling genes of different samples at specific time points. Each row represents a gene and each column represents a sample. Red means a higher expression level; green means lower expression levels. (C–E) Metascape analysis of significantly rhythmically expressed genes for each group. (C) Heatmap of the top 20 enriched terms, colored by P values that correspond to the length of the bars. (D) Network plot of the relationships among enriched terms. Each node represents an enriched term, colored by its cluster ID, and the 0.3 kappa score was applied as the threshold to cast the tree into term clusters. (E) Protein-protein interaction network analysis of significantly rhythmically expressed genes. *P* < .0001.

CLOCK, BHLHE40, NPAS, and BMAL1 were eminent in the control group, whereas the Krüppel-like family of transcription factors (KLFs), such as KLF3, KLF6, KLF9, KLF5, and KLF14, were predominant in the PCOS group (Supplemental Fig. 1C). In support of a previous study (37), our TF-binding-site prediction results indicated that the potential binding sites of CLOCK were +407 bp to +416 bp in STAR and +9 bp to +18 bp in PPARGC1A (Supplemental Fig. 1D).

The Disruption of Ovarian GC Biorhythm in PCOS

Next, by means of performing RNA-seq at various time points over 24 hours, we clarified the mechanism underlying the cell-autonomous biorhythm of ex vivo GCs that modulates ovarian function. Freshly harvested human GCs were primarily cultured and studied in a circadian fashion, and mRNA was extracted throughout extrapolated day/night periods to mimic the physiologic sleep-wake cycle (31).

First, the volcano plots of differential mRNA expressions between the PCOS and control groups clearly showed a gradual change at various time points (Figs. 2A–2E). The total numbers of up-regulated genes were 571, 434, 243, 200, and 202, and the numbers of down-regulated genes were 297, 205, 168, 178 and 180, at five time points, respectively (Fig. 2F).

We analyzed the temporal expression profiles of clock genes and found that most core clock genes were expressed in primary GCs from both groups except RORA and RORC. Some oscillations of core clock genes were largely changed in the PCOS group compared with the control group, such as NFIL3 and CRY1. And several clock genes showing strong rhythms in the GCs of control subjects, such as REVERBB, CRY2, BMAL1, and BHLHE40, oscillated in a circadian manner but with lower amplitudes in GCs of PCOS patients (Fig. 2G).

Then we identified rhythmically expressed genes in GCs with the use of RAIN and found 4,466 (14% of the total genes) in the control group and 5,045 genes (16% of the total genes) in the PCOS group. Using a P value threshold of 0.0001, we further obtained a cluster of strongly rhythmic genes for each group illustrated in a Venn diagram, and only 96 genes (28% for the control group, 23% for the PCOS group) overlap for both groups (Fig. 3A). As shown in Figure 3B, the heatmaps of hierarchical clustering analyses facilitate the visualization of the altered genome-wide rhythmic expression pattern at various time points in GCs of the PCOS group compared with the control group.

Metabolic Dysfunction Highlighted by Differential Cyclic Genes in GCs of PCOS

Furthermore, to comprehensively survey the functional associations among the differential cyclic genes for each group, metascape analysis was carried out with these databases: KEGG functional sets, KEGG pathway, GO biological processes, GO molecular functions, Immunologic signatures, Canonic pathways, Oncogenic signatures, Reactome gene sets, and CORUM. For the control group, metascape results were dominated by functional categories related to immune function, including M4531 (GSE22886_UNSTIM_VS_IL2_ STIM_NKCELL)DN), M3135 (GSE11864_CS F1_IFNG_IN_MAC_DN), and M6158 (GSE1740_MCSF_ VS_MC_SF_AND_IFNG_DAY2_DERIVED_MACROPHAGE_ WITH_IFNA_STIM_UP). On the other hand, the strongly rhythmic genes for the PCOS group mainly involve biological processes such as programmed cell death, protein secretion, and carbon metabolism (Fig. 3C). To capture the relationship among the significant functional terms, a network plot, colored by cluster ID (Fig. 3D) and P value (Supplemental Fig. 3A, available online at www.fertstert.org), was rendered based on kappa-statistical similarities among their gene memberships. An obvious difference of relationship among functional terms exists between the PCOS and control groups. Analysis of PPI networks and Molecular Complex Detection networks suggests that the interaction among proteins of women with PCOS dramatically differs from that of control women (Fig. 3E; Supplemental Fig. 3B).

To further investigate the alteration of rhythmically expressed genes of women with PCOS compared with control women, we classified all cyclic genes into two clusters based on their expression patterns (Supplemental Fig. 4A, available online at www.fertstert.org). For both clusters 1 and 2, the expression pattern of cyclic genes differed most at 11:00 AM between the PCOS and control groups. Using the strongly rhythmic transcribed genes, GO terms and KEGG pathways analyses were computed to understand the detailed functions. For KEGG analysis, group-specific pathways at the significant level of each cluster were sorted, and the results are shown in Supplemental Figure 4B. Interestingly, in cluster 1, we found pyrimidine and purine metabolism for the control group, while we found a series of pathways for the PCOS group such as the vascular endothelial growth factor signaling pathway and Ras signaling pathway. In cluster 2, steroid biosynthesis was the most enriched pathway for the control group, whereas the data from the PCOS group emphasized carbohydrate metabolism, including the pentose phosphate pathway, tricarboxylic acid (TCA) cycle, and glycolysis/gluconeogenesis. For GO enrichment analysis, we ranked these functional annotations based on the number of genes with significant differences noted for specific GO terms (gene number), and the top 20 terms of each cluster were selected and are listed in Supplemental Tables 3–6 (available online at www.fertstert.org). Strikingly, in cluster 1, we found that cytoskeleton, cell junction, and nuclear membranes were enriched for the control group, while mitochondrion, regulation of apoptotic processes were enriched for the PCOS group. In cluster 2, extracellular exosome and steroid metabolic process were the main GO terms for the control group, whereas ATP binding and mitochondrion were enriched for the PCOS group.

DISCUSSION

This study spotlights the link between circadian rhythm and PCOS by providing the first evidence of genome-wide disruption of the circadian rhythm over 24 hours in GCs of women with PCOS.

Despite emerging evidences of a high prevalence of sleep disorders in patients with PCOS (38, 39), there are limited...
epidemiologic data concerning the association between PCOS and circadian disruption by sleep disturbances or night shift work (40). Notably, in the present large population-based multicenter survey, there was a significant association between long-term exposure to rotating night shift work and PCOS. However, further verification is required by evaluating individuals of different ethnicities with the use of a prospective observational technique. Given that PCOS is associated with increasing occurrence of sleep disorders causing clock gene dysfunction, for example, obstructive sleep apnea mediated by insulin resistance (41), we excluded all individuals who were known to suffer from sleep disorders in our work.

Night shift work induces desynchronization between the circadian system and the outside world, and exposure to bright light at night during both real and simulated night work alters markers of the central and peripheral clocks (42). There is emerging evidence that urinary metabolites of melatonin, such as 6-sulphatoxymelatonin, are elevated in women with PCOS, particularly at night (14, 43). The serum levels of melatonin were elevated in the PCOS group at 4:00 AM, 8:00 AM, and noon (12, 13). In contrast, a reduced concentration of melatonin was reported in follicular fluid from women with PCOS (44, 45). In the present study, we found no difference between the PCOS and control groups in serum melatonin levels between 8:00 AM and 10:00 AM, regardless of any previous experience of night shift work according to the population-based survey, nor in the rat experiments (Figs. 1A, 1B, and 1F). However, we found a later melatonin peak in PCOS-model rats, which is supported by a recent report of a later melatonin offset in the morning in girls with PCOS and obesity (46).

In the evening, CRH and ACTH levels reached a peak in control rats, but a nadir in PCOS rats; furthermore, morning circadian misalignment of both CRH and ACTH were identified in PCOS rats (Figs. 1G and 1H). Nevertheless, the circadian rhythm of CRH does not account for rhythms of its downstream hormones in the hypothalamic-pituitary-adrenocortical axis (15).

The rhythms of clock gene expression are limited to antral GCs and luteal cells (47). Both core clock genes and clock-controlled genes involved in primary GC functions, such as steroidogenesis, are expressed in an oscillating manner and regulated by endocrine signals in vivo and ex vivo (21, 48). Consistent with a recent report of attenuated expression of the core clock gene BMAL1 in GCs of PCOS patients (49), we found that the oscillations of some core clock genes were attenuated in PCOS patients (Fig. 2A). Furthermore, we observed, for the first time, genome-wide disruption of circadian rhythm in the ovaries of women with PCOS (Fig. 2B). Among the differentially rhythmically expressed genes in GCs between the PCOS and control groups, our KEGG analysis highlighted metabolic disorders, including carbohydrate, amino acid metabolism, and the TCA cycle (Fig. 3C; Supplemental Fig. 4B).

Although all of the PCOS patients and control subjects included in the present study were free of endocrine- and metabolism-related disorders, our inspection of rhythmic gene clusters in GCs revealed the pentose phosphate pathway and pyruvate metabolism of PCOS (Supplemental Fig. 4B), indicating expression alterations of rhythmic genes involved in ovarian carbohydrate metabolism. In support of this observation, the pentose phosphate pathway, pyruvate metabolism, and TCA cycle were previously found to be essential to oocyte maturation but were impaired under the exposure of excess androgen or in PCOS (50, 51). It is also confirmed by another study that provided evidence with the use of plasma metabolomics of enhanced glycolysis, disturbed amino acid metabolism, and inhibited TCA cycle in women with PCOS (52). However, because the primary human GCs for our ex vivo study were collected from three infertile women with PCOS and three infertile women with tubal blockage as control subjects, the conclusion seems relatively limited. In the present study, the GCs were collected on the day of ovarian ovum retrieval after GnRH and hCG treatment; however, the total dosages of GnRH for the women with PCOS were much lower than for the control women, which may also potentially bias the conclusion. Therefore, it is of significance to further confirm our findings in unstimulated and nonluteinized GCs across various ethnic groups with larger sample size in future.

CONCLUSION

There is a significant association of night shift work with PCOS, and genome-wide chronodisruption exists in ovarian GCs.

REFERENCES


