Association of chronotype and depressive symptoms in Chinese infertile population undergoing assisted reproductive technology

Danni Wang[1], Fei Jiang[1], Mengli Zhu[1], Yuedi Jia[1], Xiaohuan Song[1], Wang Jieyu[2], Qianhua Xu[2], and Guiying Luo[2]

[1]Anhui Medical University School of Public Health
[2]First Affiliated Hospital of Anhui Medical University Reproductive Medicine Center

September 25, 2023

Abstract

Objective: To assess the association between chronotype and depressive symptoms in an infertile population undergoing assisted reproductive technology. **Design:** Cross-sectional study. **Setting:** The First Affiliated Hospital of Anhui Medical University Reproductive Medicine Center in Hefei, China. **Population:** 1022 infertile patients who sought assisted reproductive technology at the Center between August and October 2022, were assessed for inclusion in this study. **Methods:** On the day of ovulation injection, we collected socio-demographics by inviting infertile patients to complete questionnaires and assessed the subjects' chronotypes through the Morning and Evening Questionnaire-5 Items (MEQ-5). **Main Outcome Measures:** Depressive symptoms, assessed with validated Patient Health Questionnaire-9 (PHQ-9) and expressed as PHQ-9 scores. **Results:** Overall, 9%, 68%, and 22% of participants were categorized as evening, neutral, and morning chronotypes, respectively. There were significant differences between chronotype on levels of depressive symptoms, subjects with morning chronotype (odds ratios = 0.32, 95% confidence intervals: 0.18-0.57) and neutral chronotype (odds ratios = 0.47, 95% confidence intervals: 0.28-0.77) had lower odds of depressive status, compared to those with evening chronotype, but no partner effect of chronotype was found (p >0.05, respectively). **Conclusions:** Our findings suggest that morning and neutral chronotypes may be significantly associated with a lower likelihood of depressive symptoms. In addition, the effect of earlier chronotypes in men on depressive symptoms in women warrants relevant clinical attention when considering treatment.

Introduction

Infertility is a reproductive system disease that is characterized by the inability to achieve a clinical pregnancy after 12 or more months of regular unprotected sexual intercourse [1]. According to epidemiological surveys, the incidence of infertility is rising and trending younger, affecting 186 million couples worldwide[2], accounting for about 10%-15% [3], with a prevalence of 25% among couples of reproductive age in China[4], which has become the third most serious disease affecting human health after cardiovascular diseases and tumors[5]. With the implementation of China's fertility policy, infertile individuals and couples are under pressure from society, family, and the treatment process due to the long-term inability to have children normally, causing great psychological burden and social-emotional distress, which largely affects the physical and mental health of infertility patients [6, 7].

Depression, one of the most prevalent mental disorders, is manifested by persistent low mood, affects 4.4% of the global population proportionally, or approximately 322 million people [8], and is the second leading cause of disability worldwide[9]. Currently, it is well documented that depression is closely associated with an increased risk of developing multiple metabolic syndromes [10], including obesity[11], hypertension [12], and immuno-metabolic dysregulation [13], resulting in long-term adverse health outcomes. However, regarding the comorbidity phenomenon of depression and infertility, it is unclear whether this simply reflects shared

risk factors. There is speculation that depression may be a cause of infertility, its consequence, or both[14]. A previous study reported a significant association between depression and treatment outcomes in assisted reproductive technology (ART) [15]. As a matter of fact, depression may be even more prevalent in infertile patients compared to the general population of reproductive age [14], considering the long course of infertility, complex etiology, many cooperating factors, high treatment costs, cumbersome treatment process, and the influence of social culture and traditional beliefs. There is growing evidence that depression may be associated with multiple factors, not only risk factors from social aspects but also individual-level lifestyle factors, such as circadian rhythm disturbances, which may play a significant role in the onset and progression of depression and the overall severity of depressive symptoms [16].

Accumulating evidence suggests that circadian rhythm disturbances are associated with a number of adverse health outcomes[17], including but not limited to obesity[18], type 2 diabetes [19], cardiovascular diseases [20], reproductive functions[21], psychiatric disorders [22], and cancer [23]. The chronotype is generally considered to be one of the manifestations of circadian rhythms, and it represents the behavioral pattern of organizing events during the 24 hours of a day, especially referring to the subjective preference of individuals for sleep-wake times [24, 25], which can be divided into morning chronotype, neutral chronotype, and evening chronotype[26]. In previous studies, it was agreed that evening chronotype was a risk factor for mental health and that individuals with morning chronotype were at lower risk of developing emotional problems such as anxiety and depression compared to evening chronotype[27]. With the increasing prevalence of infertility, more and more researchers are also focusing on circadian rhythm variations in infertile individuals and have found that worse sleep quality and evening chronotype are more common in infertile populations compared to those with fertility [28]. However, it is noteworthy that two prospective studies exploring the onset and progression of infertility from a chronotype perspective suggest suggested that morning chronotype may be a risk factor for fertility[29, 30].

To be sure, it is necessary to examine the impact of morning chronotype on depression during infertility treatment. Since infertility is a more specific reproductive disorder, although not fatal, it can have a negative impact on the individual and the spouse, or even the entire family. Depressive symptoms, a major type of depression, without timely intervention are likely to develop into depression. Therefore, exploring the causes of depression is essential to promote health and function throughout the life course. To the best of our knowledge, there is a lack of clarity about the relationships between chronotype and depressive symptoms during infertility treatment. Accordingly, the objectives of this study were (1) to examine the effect of sleep type on the depressive symptoms in subjects undergoing infertility treatment. and (2) to assess the partner effect of spouses' chronotype on each other's depressive symptoms.

Materials and methods

Participants

From August to October 2022, we conducted this cross-sectional study among infertile couples in an artificial endometrial preparation from the Center for Reproductive Medicine, First Affiliated Hospital of Anhui Medical University (Hefei, Anhui, China). Those patients who met the diagnostic criteria for infertility and were actively seeking treatment were included. Subjects with a history of psychiatric disease, no embryo transfer, comorbidities such as hypertension, diabetes, or kidney disease, or voluntarily withdrew were excluded (n = 152). In this study, baseline data were collected on the day of the ovulation injection through a questionnaire that the subjects scanned a QR code using a mobile application to access the questionnaire and completed it under the constant supervision of highly trained research staff. The content of the questionnaire included general demographic characteristics (age, sex, ethnicity, education level, income, occupation, and marital status), behavioral lifestyle (smoking or passive smoking, alcohol and coffee consumption, Sleep, psychological state, physical activity, and dietary habits), family and social relationships (social capital, family power and reproductive quality of life) and fertility treatment history (parity, gravidity, history of preterm birth and abortion, infertility treatment timing and causes). Ultimately, after subjects with missing data were excluded (n = 44), a total of 1,022 infertile patients were included in the final analysis. The study protocol was approved by the Ethics Committee of Anhui Medical University (No. 20200961) in accordance with the

guidelines of the Declaration of Helsinki and international ethical standards. All invited participants have obtained a written informed consent form to participate in the study (**Figure S1**).

Chronotype assessment

At present, the most widely used circadian rhythm scale is the Morning and Evening Questionnaire, of which the Morning and Evening Questionnaire-19 Items (MEQ-19) was first proposed by Horne and Ostberg [31]. In order to quickly screen the chronotypes, we used the Morning and Evening Questionnaire-5 Items (MEQ-5), which was extracted from the MEQ-19 by Adan and Almiralli through statistical modeling [32]. It is proved to have promising psychometric properties, reliability and validity, where the Cronbach's alpha coefficient for our study was 0.703. Due to the limited number of individuals within the lowest or highest chronotype score group, the chronotype of subjects were divided into evening chronotype (4-11 score), neutral chronotype (12-17 score), and morning chronotype (18-25 score) in current study based on the MEQ-5 score.

Depressive symptoms assessment

The participants were also asked to report their psychological status over the past week through the Patient Health Questionnaire-9 (PHQ-9). It is a simple and effective self-rating scale for the depressive disorder based on the diagnostic criteria of the American Psychological Association [33], which has been demonstrated to have good reliability and validity by previous studies [34], with Cronbach's alpha coefficient being 0.910 in the present study. It contains 9 items with an overall score ranging from 0 to 27, higher scores indicate more severe depressive symptoms. In the present study, the total score was divided into a dichotomous variable, score ¿4 points indicate mild and above depressive symptoms, and conversely no depressive symptoms.

Covariates

All covariates were derived from self-reported baseline questionnaires. We searched the previously relevant literature and used a directed acyclic graph to select potential confounders[35](**Figure S2**). Specifically, the following covariables are considered in the analysis: age ([?]29, 30-34, and [?]35 years), sex (male, female), annual income (<30,000, 30,000˜60,000, [?]60,000), education (middle school or below, high/ vocational school, college degree or above), passive smoking (never, occasionally, frequently), physical activity (low, moderate, vigorous), living children (yes or no), infertility treatment time ([?]6, 7˜12, 13˜24, >24 month), cause of infertility (male, female, both, and unexplained), frequency of insomnia (never, occasionally or [?]3 per month, [?]4 per month), nocturnal wake frequency (never, occasionally, [?]1 per night), daytime napping (never, <1 and [?]1h), social jetlag (<1 and [?]1h), and nighttime sleep duration (<8 and [?]8h).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median (P25, P75), and categorical variables were expressed as percentages. We compared the distribution of participants with different demographic characteristics by chronotypes (morning chronotype, neutral chronotype, and evening chronotype) using t-tests and analysis of variance (ANOVA) for continuous variables or Chi-square tests and Fisher's exact test for categorical variables. Binary logistic regression models were used to examine the relationship between chronotype and depressive symptoms during infertility treatment. Hypothesis testing was performed to ensure that the assumptions of the regression model were met. Since the missing values are few, we handle it by deleting the rows.

We used the evening chronotype as the reference group in the binary logistic regression models to assess the odds ratios (ORs) and 95% confidence intervals (CIs) for chronotype on the depressive status and adjusted for possible confounding variables in the final model, including age, sex, annual income, education, passive smoking, physical activity, living children, infertility treatment time, cause of infertility, frequency of insomnia, nocturnal wake frequency, daytime napping, social jetlag, and nighttime sleep duration. Trend tests were performed using the median of the different categories of chronotypes scores as a continuous variable. Restrictive cubic splines with five knots were conducted to characterize the dose-response curve between sleep and depressive symptoms. We also tested the interaction effect and estimated the relationship between chronotype and odds of depressive symptoms by stratification of the covariates above. A sensitivity analysis

was applied to test the robustness of the results. As well, we utilized the Actor- Partner Interdependence Mode (APIM) approach to explore the partner effect of own chronotype on the spouse's depressive symptoms, as recommended by Kenny et al [36]. In our study, the actor effect refers to the influence of a person's chronotype on his/her own depressive symptoms, and the partner effect refers to the influence of a person's chronotype on his/her spouse's depressive symptoms. The APIM framework for a husband-wife dyad is also described in **Figure S3** .

All the above statistical analyses were carried out using R software version 4.1.0 (University of Auckland, Auckland, New Zealand), SPSS version 23.0 (SPSS, Chicago, IL, USA), and Mplus software (version 8.3). In current study, all statistical tests were double-tailed and the level of significance was p -value <0.05 which was considered statistically significant.

Results

General Characteristics of Participants across chronotype

The characteristics of the 1022 (96.2%) subjects included in present study are shown in **Table 1** . Overall, participants included a total of 608 females (59.5%) and 514 males (40.5%), with 44.7% (n = 457) of individuals aged 30 to 34 years. According to the MEQ scores, approximately 9.39% of the participants were identified as evening chronotype, while 68.2% were neutral chronotype and 29.06% were morning chronotype. Significant differences were found for age, passive smoking, education, annual income, frequency of insomnia, daytime napping, and living children compared to these three groups (all p <0.05). Specifically, participants in the evening chronotype were more likely to be younger, to be free of passive smoking, had a high or vocational school education level, a higher annual income, more frequent insomnia, never daytime napping, lower level of physical activity as well longer sleep and social jetlag times and shorter infertility treatment times. In addition, we observed that 527 individuals reported mild and above depressive symptoms, representing 51.6% of the total subjects, with a trend toward a decrease in depressive symptoms as the chronotype from evening to neutral and then to morning. The characteristics of participants according to depressive phenotype are shown in **Table S1** .

Binary logistic regression models

We assessed the association of chronotype with the risk of depressive status during infertility treatment, as shown in**Table 2** . Compared to subjects in the evening chronotype group, neutral chronotype (OR = 0.45, 95% CI: 0.28-0.72, P trend <0.001) and morning chronotype (OR = 0.29, 95% CI: 0.18-0.49, P trend <0.001) were significantly associated with lower odds of depressive symptoms. Similarly, after adjusting for confounders, neutral chronotype (OR = 0.47, 95% CI: 0.28-0.77, P trend <0.001) and morning chronotype (OR = 0.32, 95% CI: 0.18-0.57, P trend <0.001) types were still associated with lower odds of depressive status, although this risk reduction was attenuated. For each standard deviation increase in MEQ-5 scores, the adjusted ORs (95% CIs) were 0.69 (0.59-0.79, P <0.001) for depressive symptoms.

Test for nonlinear association between MEQ-5 scores and Depressive symptoms

In addition, we examined the non-linear dose-response relationship between chronotype (MEQ-5 score) and risk of depressive symptoms by restrictive cubic splines, as shown in Figure 2. After adjusting for age, sex, annual income, education, passive smoking, physical activity, living children, infertility treatment time, cause of infertility, frequency of insomnia, nocturnal wake frequency, daytime napping, social jetlag, and nighttime sleep duration, we found that the odds of depressive symptoms appeared to decrease with increasing MEQ-5 scores (P overall < 0.001); however, no nonlinear trend was observed (P non-linear = 0.526) (**Figure 1**).

Stratified and sensitivity analyses

In stratified analysis, we did not find any differential association of chronotype with odds of depressive symptoms, according to age, sex, annual income, education, passive smoking, physical activity, living children, infertility treatment time, cause of infertility, frequency of insomnia, nocturnal wake frequency, daytime

napping, social jetlag, and nighttime sleep duration (all p for interaction > 0.05). As illustrated in **Figure 2** .

In addition, we performed repeated analyses to test the robustness of the results by excluding individuals who worked shift or night, the association of morning and neutral chronotypes with the risk of depressive status were similar to the main analysis (OR = 0.51, 95% CI: 0.30-0.87, P trend <0.001; OR = 0.32, 95% CI: 0.18-0.58, P trend <0.001, respectively). As illustrated in **Table S2** .

APIM analysis

We screened 397 infertile couples among 1022 subjects for inclusion in the APIM with distinguishable dyads. The results showed that both female and male chronotype exerted an actor effect on their own depressive symptoms (β = -0.514, p <0.001; β = -0.228,p <0.045, respectively). However, contrary to our expectations, our study did not observe a strong partner effect of female/male chronotype on spouse depressive symptoms. Specifically, the earlier chronotype for husband had a detrimental effect on wife's depressive symptoms (β = 0.145, p = 0.200), yet the earlier chronotype for the wife had a protective effect on husband's depressive symptoms (β = -0.074, p = 0.577), but neither was statistically significant (**Table 3**).

Discussion

Main findings

In this cross-sectional study of 1022 Chinese infertility patients, we observed that morning and neutral chronotypes individuals were less likely to experience depressive symptoms during assisted reproductive treatment compared to evening chronotype, based on comparative analysis of chronotype and PHQ-9 scores, and this protective association appeared stronger in persons aged [?]30 years and among individuals with nighttime sleep duration <8h or social jetlag [?]1h. As far as we know, this is the first observational study to investigate the relationship between chronotype and depressive status among the infertility population who underwent assisted reproductive technology treatment. However, findings should be interpreted cautiously as the chronotype were based on self-reported MEQ-5 scores that may be misclassified.

Strengths and limitations

Several strengths of the current study in regard to the methods and design, including selected infertile couples with more fully displayed psychological characteristics as research objects, which ensured the credibility of the research results to a certain extent. Additionally, in terms of exposure assessment, we systematically collected sleep characteristics through the 22-item Sleep Factor Questionnaire (SFQ)[37]. Meantime, we also adjusted for other sleep factors that may influence the outcome for incident depression in the analysis. We were also the first group to explore the relationships between chronotype and depressive symptoms in infertile couple, although no partner interdependence effects may not be found in the relationships.

Whereas, some limitations to the present study should be noted. First, since our design was cross-sectional studies rather than longitudinal studies or randomized clinical trials, which may describe associations, but are limited to causal inference. However, this study can still provide clues about risk factors for depressive symptoms and may provide the scientific basis for future studies. Second, participants' chronotype and depressive symptoms were self-reported on the day of oocyte retrieval based on validated scales with good reliability and validity, which still may lead to bias and reduce the power of our evidence in some respects. Third, the study was based on couples undergoing infertility treatment, and the findings may not be directly generalizable to other populations. Also, the data on sleep characteristics in this study was based on the past six months, which may not reflect long-term sleep habits. Also, this study lacks objective indicators to explain the biological mechanism behind the relationship between chronotype and depressive symptoms in infertile couples, future studies need to be further verified.

Interpretation

Previous studies of chronotype and depression in infertility patients were quite limited, First, most studies agree that chronotype may be significantly associated with negative affect, including the general population,

the elderly, pregnant women, children, or adolescents, which is consistent with our findings in the infertility population. Similar findings were observed in a Mendelian randomization study, where earlier diurnal preference was shown to be associated with a 23% lower risk of major depressive disorder [38]. Secondly, an animal study of cortisol-induced depression-like behavior, suggested that circadian rhythms may cause or predict episodes of depression[39]. However, a recently conducted longitudinal study showed that chronotype did not predict the duration of depression or anxiety disorders [40]. The above differences may be due to the study subjects, whether the findings from animal experiments are directly analogous to humans, as well as how well chronotype predict the course of depressive illness, further clinical trials or cohort studies are still needed to investigate these aspects of health effects. Thirdly, Chronotype not only to be associated with a depressive disorder diagnosis but also with the severity of the disorder[40]. Interestingly, a 7-year follow-up study showed that changes in chronotype was only associated with the severity of depressive symptoms but not with anxiety symptoms [41]. Next, the independent role of chronotype has also been reported. A study found that daytime sleepiness and sleep debt mediated the effects of evening chronotype preferences in young adult college students increasing the risk of depression and anxiety, but not in the general population of young adults [42]. Moreover, studies have confirmed that evening chronotype have a higher risk of depression than other chronotypes and that the association exists independently of perceived stress, poor sleep, and insufficient sleep duration[43, 44]. Undoubtedly, the findings of these studies reinforce the importance of studying the role of chronotype in depression. Additionally, chronotype studies in infertility populations have shown that morning chronotype may be the risk factor for IVF-ET outcomes, with the lowest rates of clinical pregnancy and live birth and the highest rate of miscarriage [30]. Another prospective study also showed that mid-sleep time (MST) earlier than 2:21 a.m. (<2:21 a.m.) or later than 3:00 a.m. ([?]3:00 a.m.) was negatively associated with fertilization rates [29]. The effect of chronotype on health varies across studies with different outcome variables observed. Although morning chronotype was shown to be a protective factor for depressive symptoms in our study, it may also be a risk factor for physical health, more studies with larger sample sizes are necessary to determine the effect of time type on the infertility population.

Our results confirm and extend previous epidemiological studies showing the sex-specific effects of chronotype on depressive status. A study among 5,550 non-shift working adults in Korea found that late chronotype was associated with a 2.9-fold increased risk of depression in women, but not in men [45]. Our findings are broadly consistent with previous studies and extend these observations further into the infertility population. Specifically, we found through APIM that own depressive symptoms may not be influenced by the chronotype of the partner. Notably, our findings also showed a significant interaction between age and chronotype on the odds of developing depressive symptoms, whereby the health benefits of depressive symptoms were greater for morning chronotype and neutral chronotype in participants aged [?]30 years. The chronotype depend on genetic and age-related factors, and it becomes earlier as aging progresses[46], generally adolescents and young adults show the evening chronotype [47]. A study with a predominantly rural population reported that mild to severe depression was significantly associated with later chronotype and higher social jetlag (> 2h), especially in ages 31-40 years[48], which is broadly consistent with our study. In addition, we observed that sleep deprivation and high social jetlag may be another cause of depressive symptoms, morning chronotype and neutral chronotype produced stronger health benefits for depressive symptoms in subjects with high social jetlag ([?]1h) and sleep deprivation (<8h). A large number of epidemiological studies have explored the relationship between sleep deprivation and depression, showing sleep deprivation to be an important risk factor for the development of depression and this association may be driven by the pathway of sleep disorder. Also, one recent study has shown that social jetlag is significantly associated with depression and independent of sleep debt[49]. It is partially consistent with our conjecture, but further clinical trials or cohort studies are needed to validate these health effects in the future.

Although the underlying mechanisms on the association between chronotype and depressive symptoms are poorly understood, there are possible mechanisms. First, it has been well-documented that the amygdala reactivity plays a crucial role in emotional outcomes[50]. Specifically, the later chronotype was associated with increased reactivity of the amygdala to negative affective stimuli, and similar findings have been reported in patients with depression and in high-risk populations, including highly neurotic and with family history of

depression [51]. Secondly, apart from the degree of amygdala activation related to emotional processing, there are also differences in the functional connectivity of the amygdala and dorsal anterior cingulate cortex. Horne and colleagues found significantly reduced functional connectivity between amygdala and dorsal anterior cingulate cortex in the later chronotype[51]. It means that the evening chronotype may produce a stronger emotional response to negative stimuli, which inhibits the dorsal anterior cingulate regulation to the amygdala and thus affects emotion modulation.

Conclusions

To conclude, our study found that significantly associated between chronotype and depressive symptoms in infertility patients, although there may not be a partner effect. Morning and neutral chronotypes may be effective in alleviating depressive symptoms during infertility treatment. From a clinical and public health perspective, improving the psychological status of infertility patients during treatment may benefit reproductive treatment outcomes. Also, the underlying causes of depressive symptoms may be multifactorial, and additional prospective studies are needed to validate this association as well as to identify effective strategies to promote mental health in this population.

Footnote

Author' contributions: Fei Jiang had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Danni Wang. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Fei Jiang and Danni Wang. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Fei Jiang. Obtained funding: Danni Wang. Administrative, technical, or material support: Danni Wang. Study supervision: Danni Wang.

Acknowledgments: The authors would like to thank the Center for Reproductive Medicine, First Affiliated Hospital of Anhui Medical University for their technical support for data collection. The authors would also like to thank the study participants and staff.

Institutional Review Board Statement: The Ethics Committee of Anhui Medical University approved the study protocols (No. 20200961). And the study design was confirmed in accordance with the Helsinki Declaration.

Informed Consent Statement: All participants provided informed consent before enrollment.

Funding: This work was supported by the National Natural Science Foundation of China (72004001), and grants from Anhui Provincial Scientific Research and Humanities Project of Colleges and Universities (2022AH050627).

Disclosure statement: All authors have completed the ICMJE uniform disclosure form and have no conflicts of interest to declare.

Attestation statement: The subjects in this trial have not concomitantly been involved in other randomized trials. Data regarding any of the subjects in the study has not been previously published unless specified. Data will be made available to the editors of the journal for review or query upon request.

Data sharing statement: Data will be made available on request.

Word counting: 222 words for abstract and 3784 words for text.

Number of figures and tables : 3 tables, 2 figures, 2 supplemental tables, and 3 supplemental figures.

References

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Hum Reprod. 2017 Sep 1;32(9):1786-801.

2. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015 Jul-Aug;21(4):411-26.

3. Tamrakar SR, Bastakoti R. Determinants of Infertility in Couples. J Nepal Health Res Counc. 2019 Apr 28;17(1):85-9.

4. Zhou Z, Zheng D, Wu H, Li R, Xu S, Kang Y, et al. Epidemiology of infertility in China: a population-based study. BJOG. 2018 Mar;125(4):432-41.

5. Agarwal A, Majzoub A, Parekh N, Henkel R. A Schematic Overview of the Current Status of Male Infertility Practice. World J Mens Health. 2020 Jul;38(3):308-22.

6. Cocchiaro T, Meneghini C, Dal Lago A, Fabiani C, Amodei M, Miriello D, et al. Assessment of sexual and emotional distress in infertile couple: validation of a new specific psychometric tool. J Endocrinol Invest. 2020 Dec;43(12):1729-37.

7. Kamboj N, Saraswathy KN, Prasad S, Babu N, Puri M, Sharma A, et al. Women infertility and common mental disorders: A cross-sectional study from North India. PLoS One. 2023;18(1):e0280054.

8. Friedrich MJ. Depression Is the Leading Cause of Disability Around the World. JAMA. 2017 Apr 18;317(15):1517.

9. Refisch A, Sen ZD, Klassert TE, Busch A, Besteher B, Danyeli LV, et al. Microbiome and immunometabolic dysregulation in patients with major depressive disorder with atypical clinical presentation. Neuropharmacology. 2023 Sep 1;235:109568.

10. Ferriani LO, Silva DA, Molina M, Mill JG, Brunoni AR, da Fonseca MJM, et al. Depression is a risk factor for metabolic syndrome: Results from the ELSA-Brasil cohort study. J Psychiatr Res. 2023 Feb;158:56-62.

11. Ramzi NH, Auvinen J, Veijola J, Miettunen J, Ala-Mursula L, Sebert S, et al. Depression mediates the relationship between alexithymia and obesity in the Northern Finland Birth Cohort 1966 (NFBC1966). J Affect Disord. 2023 Jun 15;331:1-7.

12. Shah RM, Doshi S, Shah S, Patel S, Li A, Diamond JA. Impacts of Anxiety and Depression on Clinical Hypertension in Low-Income US Adults. High Blood Press Cardiovasc Prev. 2023 Jun 1:1-6.

13. Toenders YJ, Schmaal L, Nawijn L, Han LKM, Binnewies J, van der Wee NJA, et al. The association between clinical and biological characteristics of depression and structural brain alterations. J Affect Disord. 2022 Sep 1;312:268-74.

14. Vo TM, Tran QT, Le CV, Do TT, Le TM. Depression and associated factors among infertile women at Tu Du hospital, Vietnam: a cross-sectional study. International journal of women's health. 2019;11:343-51.

15. Purewal S, Chapman SCE, van den Akker OBA. Depression and state anxiety scores during assisted reproductive treatment are associated with outcome: a meta-analysis. Reprod Biomed Online. 2018 Jun;36(6):646-57.

16. Monteggia LM, Kavalali ET. Circadian rhythms: Depression brought to light. Nature. 2012 Nov 22;491(7425):537-8.

17. Comas M, Solis Flores A, Lovato N, Miller CB, Bartlett DJ, Grunstein RR, et al. The Relationship between Anxiety, Subjective and Objective Sleep, Chronotype and Circadian Rhythms with Depressive Symptoms in Insomnia Disorder. Brain Sci. 2023 Apr 4;13(4).

18. Li Y, Ma J, Yao K, Su W, Tan B, Wu X, et al. Circadian rhythms and obesity: Timekeeping governs lipid metabolism. J Pineal Res. 2020 Oct;69(3):e12682.

19. Peng X, Fan R, Xie L, Shi X, Dong K, Zhang S, et al. A Growing Link between Circadian Rhythms, Type 2 Diabetes Mellitus and Alzheimer's Disease. Int J Mol Sci. 2022 Jan 3;23(1).

20. Mentzelou M, Papadopoulou SK, Papandreou D, Spanoudaki M, Dakanalis A, Vasios GK, et al. Evaluating the Relationship between Circadian Rhythms and Sleep, Metabolic and Cardiovascular Disorders: Current Clinical Evidence in Human Studies. Metabolites. 2023 Mar 1;13(3).

21. Caetano G, Bozinovic I, Dupont C, Léger D, Lévy R, Sermondade N. Impact of sleep on female and male reproductive functions: a systematic review. Fertility and sterility. 2021 Mar;115(3):715-31.

22. Codoner-Franch P, Gombert M, Martinez-Raga J, Cenit MC. Circadian Disruption and Mental Health: The Chronotherapeutic Potential of Microbiome-Based and Dietary Strategies. Int J Mol Sci. 2023 Apr 20;24(8).

23. Lotti S, Dinu M, Colombini B, Amedei A, Sofi F. Circadian rhythms, gut microbiota, and diet: Possible implications for health. Nutr Metab Cardiovasc Dis. 2023 May 14.

24. Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. Epidemiology of the human circadian clock. Sleep Med Rev. 2007 Dec;11(6):429-38.

25. Senesi P, Ferrulli A, Luzi L, Terruzzi I. Chrono-communication and cardiometabolic health: The intrinsic relationship and therapeutic nutritional promises. Frontiers in endocrinology. 2022;13:975509.

26. Park H, Lee HK, Lee K. Chronotype and suicide: The mediating effect of depressive symptoms. Psychiatry Res. 2018 Nov;269:316-20.

27. Antypa N, Vogelzangs N, Meesters Y, Schoevers R, Penninx BW. Chronotype Associations with Depression and Anxiety Disorders in a Large Cohort Study. Depress Anxiety. 2016 Jan;33(1):75-83.

28. Ozcelik C, Varli B, Gokce A, Takmaz T, Cetin C, Ozcan P. Evaluation of chronotype and sleep quality in infertile population and comparison with fertile population: a cross-sectional study. J Psychosom Obstet Gynaecol. 2023 Dec;44(1):2148523.

29. Yao QY, Yuan XQ, Liu C, Du YY, Yao YC, Wu LJ, et al. Associations of sleep characteristics with outcomes of IVF/ICSI treatment: a prospective cohort study. Hum Reprod. 2022 May 30;37(6):1297-310.

30. Liu Z, Zheng Y, Wang B, Li J, Qin L, Li X, et al. The impact of sleep on in vitro fertilization embryo transfer outcomes: a prospective study. Fertility and sterility. 2023 Jan;119(1):47-55.

31. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4(2):97-110.

32. Adan A, Almirall H. The influence of age, work schedule and personality on morningness dimension. Int J Psychophysiol. 1992 Mar;12(2):95-9.

33. Kroenke K. PHQ-9: global uptake of a depression scale. World Psychiatry. 2021 Feb;20(1):135-6.

34. Yin L, Teklu S, Pham H, Li R, Tahir P, Garcia ME. Validity of the Chinese Language Patient Health Questionnaire 2 and 9: A Systematic Review. Health Equity. 2022;6(1):574-94.

35. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. International journal of epidemiology. 2016 Dec 1;45(6):1887-94.

36. Kenny DA, Kashy DA, Cook WL. Dyadic data analysis. 2006 [cited; Available from:

37. Yang WS, Wang X, Deng Q, Zhao H, Fan WY. Sleep duration and breast cancer risk in the breast cancer detection demonstration project follow-up cohort: true associations or bias? British journal of cancer. 2015 May 26;112(11):1838-9.

38. Daghlas I, Lane JM, Saxena R, Vetter C. Genetically Proxied Diurnal Preference, Sleep Timing, and Risk of Major Depressive Disorder. JAMA psychiatry. 2021 Aug 1;78(8):903-10.

39. Spulber S, Conti M, DuPont C, Raciti M, Bose R, Onishchenko N, et al. Alterations in circadian entrainment precede the onset of depression-like behavior that does not respond to fluoxetine. Translational psychiatry. 2015 Jul 14;5(7):e603.

40. Druiven SJM, Knapen SE, Penninx B, Antypa N, Schoevers RA, Riese H, et al. Can chronotype function as predictor of a persistent course of depressive and anxiety disorder? J Affect Disord. 2019 Jan 1;242:159-64.

41. Druiven SJM, Hovenkamp-Hermelink JHM, Knapen SE, Kamphuis J, Haarman BCM, Penninx B, et al. Stability of chronotype over a 7-year follow-up period and its association with severity of depressive and anxiety symptoms. Depress Anxiety. 2020 May;37(5):466-74.

42. Dickinson DL, Wolkow AP, Rajaratnam SMW, Drummond SPA. Personal sleep debt and daytime sleepiness mediate the relationship between sleep and mental health outcomes in young adults. Depress Anxiety. 2018 Aug;35(8):775-83.

43. Tonon AC, Carissimi A, Schimitt RL, de Lima LS, Pereira FDS, Hidalgo MP. How do stress, sleep quality, and chronotype associate with clinically significant depressive symptoms? A study of young male military recruits in compulsory service. Revista brasileira de psiquiatria (Sao Paulo, Brazil : 1999). 2020 Jan-Feb;42(1):54-62.

44. Merikanto I, Kronholm E, Peltonen M, Laatikainen T, Vartiainen E, Partonen T. Circadian preference links to depression in general adult population. J Affect Disord. 2015 Dec 1;188:143-8.

45. Kim KM, Han SM, Heo K, Kim WJ, Chu MK. Sex differences in the association between chronotype and risk of depression. Scientific reports. 2020 Oct 28;10(1):18512.

46. Taillard J, Sagaspe P, Philip P, Bioulac S. Sleep timing, chronotype and social jetlag: Impact on cognitive abilities and psychiatric disorders. Biochemical pharmacology. 2021 Sep;191:114438.

47. Keller LK, Zöschg S, Grünewald B, Roenneberg T, Schulte-Körne G. [Chronotype and depression in adolescents – a review]. Zeitschrift fur Kinder- und Jugendpsychiatrie und Psychotherapie. 2016;44(2):113-26.

48. Levandovski R, Dantas G, Fernandes LC, Caumo W, Torres I, Roenneberg T, et al. Depression scores associate with chronotype and social jetlag in a rural population. Chronobiology international. 2011 Nov;28(9):771-8.

49. Okajima I, Komada Y, Ito W, Inoue Y. Sleep Debt and Social Jetlag Associated with Sleepiness, Mood, and Work Performance among Workers in Japan. International journal of environmental research and public health. 2021 Mar 12;18(6).

50. Schiel JE, Tamm S, Holub F, Petri R, Dashti HS, Domschke K, et al. Associations Between Sleep Health and Amygdala Reactivity to Negative Facial Expressions in the UK Biobank Cohort. Biological psychiatry. 2022 Nov 1;92(9):693-700.

51. Horne CM, Norbury R. Late chronotype is associated with enhanced amygdala reactivity and reduced fronto-limbic functional connectivity to fearful versus happy facial expressions. NeuroImage. 2018 May 1;171:355-63.

Table 1. The characteristics of participants according to Morning and Evening Questionnaire score status [+].

Characteristic	Overall	Chronotypes^{++}	Chronotypes^{++}	Chronotypes^{++}	p
		Evening Chronotype	Neutral Chronotype	Morning Chronotype	
No. of Participants	1022	96(9.4)	697(68.2)	229(22.4)	

Characteristic	Overall	Chronotypes^{++}	Chronotypes^{++}	Chronotypes^{++}	p
Age (%)					0.010
[?]29	377 (36.9)	41 (42.7)	268 (38.5)	68 (29.7)	
30-34	457 (44.7)	46 (47.9)	305 (43.8)	106 (46.3)	
[?]35	188 (18.4)	9 (9.4)	124 (17.8)	55 (24.0)	
Sex (Female, %)	608 (59.5)	56 (58.3)	428 (61.4)	124 (54.1)	0.148
Education (%)					0.011
Middle school or below	302 (29.5)	24 (25.0)	190 (27.3)	88 (38.4)	
High/ vocational school	382 (37.4)	43 (44.8)	263 (37.7)	76 (33.2)	
College degree or above	338 (33.1)	29 (30.2)	244 (35.0)	65 (28.4)	
Annual incomes (%)					0.043
<30, 000	388 (38.0)	30 (31.2)	255 (36.6)	103 (45.0)	
30, 000-60, 000	242 (23.7)	23 (24.0)	178 (25.5)	41 (17.9)	
[?]60, 000	392 (38.4)	43 (44.8)	264 (37.9)	85 (37.1)	
Passive smoking (%)					<0.001
Never	326 (31.9)	49 (51.0)	220 (31.6)	57 (24.9)	
Occasionally	591 (57.8)	45 (46.9)	419 (60.1)	127 (55.5)	
Frequently	105 (10.3)	2 (2.1)	58 (8.3)	45 (19.7)	
Physical activity (%)					0.467
Low	342 (33.5)	34 (35.4)	223 (32.0)	85 (37.1)	
Moderate	341 (33.4)	31 (32.3)	244 (35.0)	66 (28.8)	
Vigorous	339 (33.2)	31 (32.3)	230 (33.0)	78 (34.1)	
Cause of infertility (%)					0.923
Male factor	179 (17.5)	14 (14.6)	126 (18.1)	39 (17.0)	
Female factor	365 (35.7)	39 (40.6)	242 (34.7)	84 (36.7)	
Both	249 (24.4)	21 (21.9)	174 (25.0)	54 (23.6)	
Unexplained	229 (22.4)	22 (22.9)	155 (22.2)	52 (22.7)	
Living children (yes, %)	162 (15.9)	9 (9.4)	105 (15.1)	48 (21.0)	0.020
Infertility treatment time					0.335
[?]6 month	342 (33.5)	40 (41.7)	223 (32.0)	79 (34.5)	
7-12 month	217 (21.2)	17 (17.7)	152 (21.8)	48 (21.0)	
13-24 month	310 (30.3)	26 (27.1)	223 (32.0)	61 (26.6)	
>24 month	153 (15.0)	13 (13.5)	99 (14.2)	41 (17.9)	
Frequency of insomnia (%)					<0.001
Never	236 (23.1)	14 (14.6)	156 (22.4)	66 (28.8)	
Occasionally or [?]3 per month	687 (67.2)	60 (62.5)	476 (68.3)	151 (65.9)	
[?]4 per month	99 (9.7)	22 (22.9)	65 (9.3)	12 (5.2)	

Characteristic	Overall	Chronotypes^{++}	Chronotypes^{++}	Chronotypes^{++}	*p*
Nocturnal wake frequency (%)					0.087
Never	158 (15.5)	23 (24.0)	106 (15.2)	29 (12.7)	
Occasionally	471 (46.1)	42 (43.8)	327 (46.9)	102 (44.5)	
[?]1 per night	393 (38.5)	31 (32.3)	264 (37.9)	98 (42.8)	
Daytime napping (%)					0.004
Never	295 (28.9)	41 (42.7)	199 (28.6)	55 (24.0)	
<1	525 (51.4)	33 (34.4)	366 (52.5)	126 (55.0)	
[?]1	202 (19.8)	22 (22.9)	132 (18.9)	48 (21.0)	
Social jetlag ([?]1 h, %)	211 (20.6)	29 (30.2)	147 (21.1)	35 (15.3)	0.009
Nighttime sleep duration ([?]8 h, %)	680 (66.5)	71 (74.0)	454 (65.1)	155 (67.7)	0.210
Depression symptoms (yes, %)	527 (51.6)	68 (70.8)	364 (52.2)	95 (41.5)	<0.001

Note: $^{+}$ Values were presented as mean ± SD or percentages; $^{++}$ Evening Chronotype: 4-11 score; Neutral Chronotype: 12-17 score; Morning Chronotype: 18-25 score.

Table 2 . Association between chronotype and depressive symptoms in infertility populations according to Morning and Evening Questionnaire score status $^{+}$.

Depressive symptoms	OR (95% CIs)	OR (95% CIs)	OR (95% CIs)	Per 1-SD	P_{trend}
	Evening chronotypes	Neutral chronotype	Morning chronotype		
No. of cases/participants	68/96	364/697	95/229		
Model 1[a]	Reference	0.45 (0.28-0.72) **	0.29 (0.18-0.49) ***	0.68 (0.60-0.78) ***	<0.00
Model 2[b]	Reference	0.45 (0.28-0.73) **	0.31 (0.18-0.53) ***	0.68 (0.59-0.78) ***	<0.00
Model 3[c]	Reference	0.47 (0.28-0.77) **	0.32 (0.18-0.57) ***	0.69 (0.59-0.79) ***	<0.00

Note: CIs, confidence intervals; ORs, odds ratios.

$^{+}$ Evening Chronotype: 4-11 score; Neutral Chronotype: 12-17 score; Morning Chronotype: 18-25 score.

* P <0.05, ** P <0.01, *** P <0.001.

[a] Model 1: did not adjust for the covariates.

[b] Model 2: additionally included age, sex, education, annual incomes, education, passive smoking, physical activity, cause of infertility, living children, and infertility treatment time.

[c] Model 3: additionally included frequency of insomnia, nocturnal wake frequency, daytime napping, social jetlag, and nighttime sleep duration.

[d] The trend test was performed by assigning medians to three categories as a continuous variable in the models.

Table 3 . Actor and partner effects of chronotype on depressive symptoms in infertile couples (n = 397 couples)

	Female	Female	Male	Male	Male	
	β (SE)	*P*	*P*	β (SE)	*P*	*P*
Actor's depressive symptoms	-0.514(0.139)	0.001	0.001	- 0.228(0.114)	0.045	0.045
Partner's depressive symptoms	0.145 (0.113)	0.200	0.200	- 0.074(0.132)	0.577	0.577

Note: β, Standardized Coefficient; SE, Standard Error

Table Legends

Table 1. The characteristics of participants according to Morning and Evening Questionnaire score status [+].

Note: [+] Values were presented as mean ± SD or percentages; [++] Evening Chronotype: 4-11 score; Neutral Chronotype: 12-17 score; Morning Chronotype: 18-25 score.

Table 2 . Association between chronotype and depressive symptoms in infertility populations according to Morning and Evening Questionnaire score status [+].

Note: CIs, confidence intervals; ORs, odds ratios. [+]Evening Chronotype: 4-11 score; Neutral Chronotype: 12-17 score; Morning Chronotype: 18-25 score. [*] P <0.05,[**] P <0.01, [***] P <0.001. [a] Model 1: did not adjust for the covariates. [b] Model 2: additionally included age, sex, education, annual incomes, education, passive smoking, physical activity, cause of infertility, living children, and infertility treatment time. [c] Model 3: additionally included frequency of insomnia, nocturnal wake frequency, daytime napping, social jetlag, and nighttime sleep duration. [d] The trend test was performed by assigning medians to three categories as a continuous variable in the models.

Table 3 . Actor and partner effects of chronotype on depressive symptoms in infertile couples (n = 397 couples).

Note: β, Standardized Coefficient; SE, Standard Error

Table S1. The characteristics of participants according to depression phenotypes [+].

Note: [+] Values were presented as mean ± SD or percentages; [++] non-Depression: 0-4 score; Depression: 5-27 score.

Note: CIs, confidence intervals; ORs, odds ratios. [+]Evening Chronotype: 4-11 score; Neutral Chronotype: 12-17 score; Morning Chronotype: 18-25 score.[*] P <0.05, [**] P <0.01,[***] P <0.001. [a] Model 1: did not adjust for the covariates. [b] Model 2: additionally included age, sex, education, annual incomes, education, passive smoking, physical activity, cause of infertility, living children, and infertility treatment time. [c] Model 3: additionally included frequency of insomnia, nocturnal wake frequency, daytime napping, social jetlag, and nighttime sleep duration.[d] The trend test was performed by assigning medians to three categories as a continuous variable in the models.

Table S2 . Sensitivity analysis on association between chronotype and depressive states among individuals without night/shifts work in infertility populations according to Morning and Evening Questionnaire score status [+].

Figure Legends

Figure 1. Visualization of the dose-response relationship between chronotype and depressive symptoms based on restricted cubic splines. Abbreviations: OR, Odds ratio; CI, confidence interval. Covariates adjusted in the models were the same as those in model 4 in**Table 2** (see **Table 2** footnote).

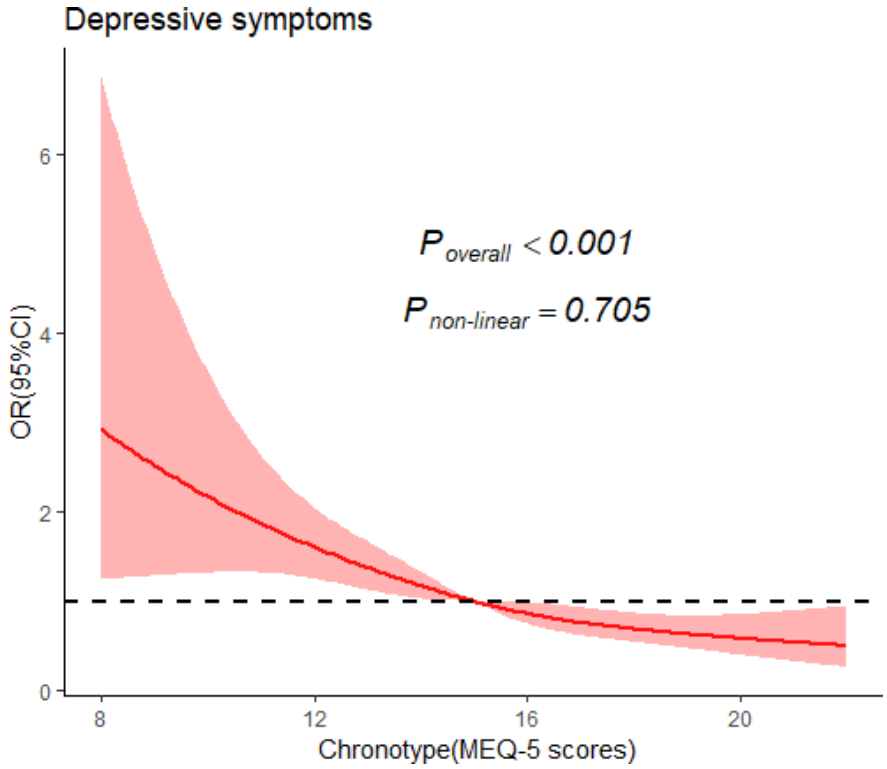
Figure 2. Stratified analysis on the association between chronotype and odds of depressive symptoms. Abbreviations: OR, Odds ratio. Covariates adjusted in the models were the same as those in model 4 in **Table 2** (see **Table 2** footnote).

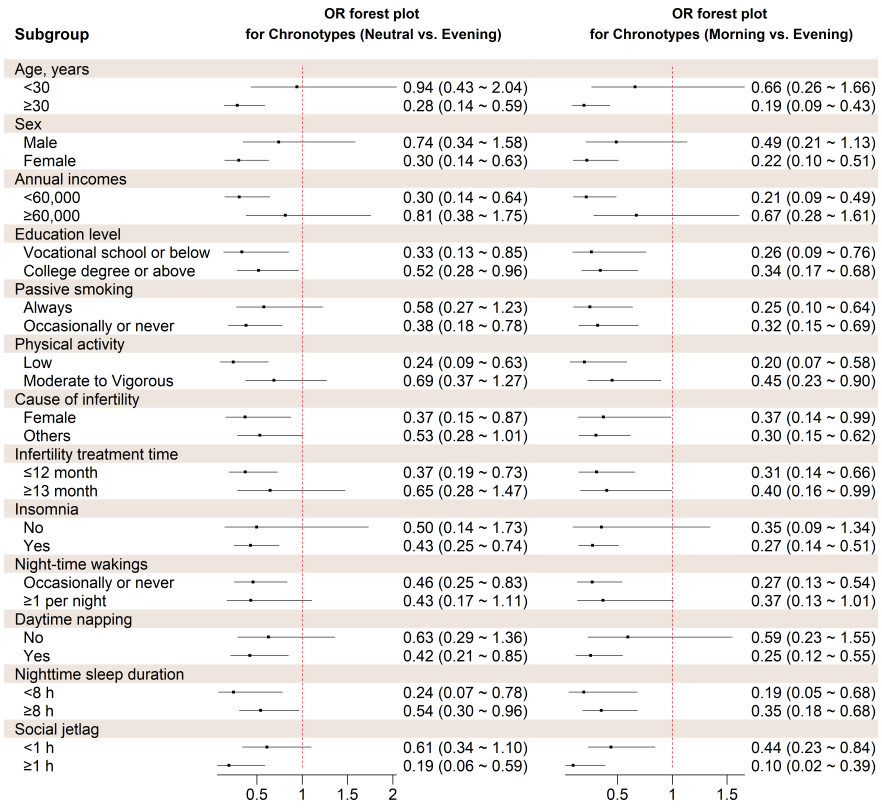
Figure S1. Flow chart of selection of participants in the analysis.

Figure S2. Directed acyclic graph for confounder selection

Figure S3. Actor–Partner Interdependence Model of chronotype and depressive symptoms in infertile couples.

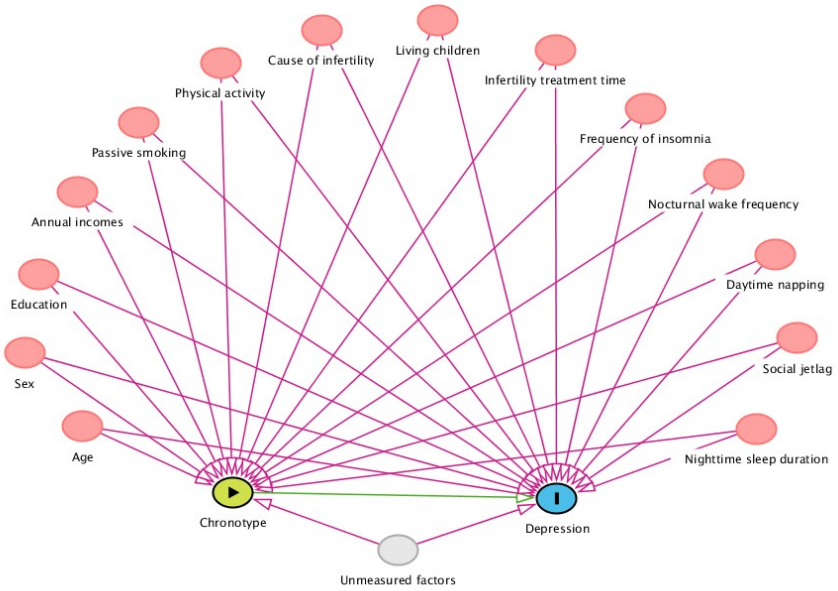
Note: Am: actor effect of female's chronotype on her own depressive symptoms; Af: actor effect of male's chronotype on his own depressive symptoms; Pfm: partner effect of female's chronotype on male's depressive symptoms; Pmf: partner effect of male's chronotype on female's depressive symptoms; Em and Ef: residual errors on depressive symptoms for female and male, respectively.





Hosted file

`Figure S1.docx` available at https://authorea.com/users/668419/articles/668302-association-of-chronotype-and-depressive-symptoms-in-chinese-infertile-population-undergoing-assisted-reproductive-technology



Hosted file

`Figure S3.docx` available at https://authorea.com/users/668419/articles/668302-association-of-chronotype-and-depressive-symptoms-in-chinese-infertile-population-undergoing-assisted-reproductive-technology

Hosted file

`Table S1.docx` available at https://authorea.com/users/668419/articles/668302-association-of-chronotype-and-depressive-symptoms-in-chinese-infertile-population-undergoing-assisted-reproductive-technology

Hosted file

`Table S2.docx` available at https://authorea.com/users/668419/articles/668302-association-of-chronotype-and-depressive-symptoms-in-chinese-infertile-population-undergoing-assisted-reproductive-technology