Study on the Impact of Immune-Related Impairment Caused by Sleep Apnea on the Reproductive System of Female Rats (Postprint)

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Abstract

Abstract

Background: Obstructive sleep apnea (OSA) is a highly prevalent clinical sleep disorder. With changes in lifestyle, its incidence continues to rise, leading to multi-system damage including cardiovascular and metabolic impairments. In recent years, it has been discovered that female patients with sleep apnea often exhibit reproductive dysfunctions such as menstrual disorders and infertility. However, the immune regulatory mechanisms of sleep apnea-induced female reproductive system damage, particularly the role of abnormal immune responses mediated by dendritic cells (DCs), still lack in-depth analysis.

Objective: To study the effects of obstructive sleep apnea-induced immune-related impairment on the reproductive system of female rats.

Methods: From 2023 to 2024, 30 female SD rats (4–6 months old) with regular estrous cycles were randomly divided into a blank control group, a short-term group, and a long-term group, with 10 rats in each group. The blank control group was raised normally for 6 weeks; the short-term group was raised normally for 3 weeks and then raised for another 3 weeks after the establishment of the sleep apnea model; the long-term group was raised for 6 weeks following the establishment of the sleep apnea model. The estrous cycle, the expression levels of estrogen receptor alpha ($ER\alpha$) and estrogen receptor beta ($ER\beta$) in ovarian tissues, and the number of follicles in ovarian tissues were analyzed for each group. Changes in DC migration capacity, the ability of DCs to stimulate allogeneic T lymphocyte proliferation (MLR), and the expression levels of Toll-like receptor 4 (TLR4) and RelB within DCs were observed. Furthermore,

the fertility of the rats in each group and the growth and development of the offspring mice were analyzed.

Results: Compared with the blank control group, the rate of estrous cycle disorder in the long-term group increased ($P < 0.05$). Compared with the blank control group, the expression levels of $ER\alpha$ and $ER\beta$ in the ovarian tissues of the short-term and long-term groups decreased ($P < 0.05$); compared with the short-term group, the expression levels of $ER\alpha$ and $ER\beta$ in the ovarian tissues of the long-term group further decreased ($P < 0.05$). Compared with the blank control group, the numbers of primordial follicles, primary follicles, and antral follicles decreased, while the number of atretic follicles increased in the short-term and long-term groups ($P < 0.05$); compared with the short-term group, the numbers of primordial, primary, and antral follicles decreased, and the number of atretic follicles increased in the long-term group ($P < 0.05$). Compared with the blank control group, the DC migration ratio decreased and MLR increased in the short-term and long-term groups ($P < 0.05$); compared with the short-term group, the DC migration ratio decreased and MLR increased in the long-term group ($P < 0.05$). Compared with the blank control group, the expression levels of TLR4 and RelB in DCs increased in both the short-term and long-term groups ($P < 0.05$); compared with the short-term group, the expression levels of TLR4 and RelB in DCs further increased in the long-term group ($P < 0.05$). Compared with the blank control group, the pregnancy rate and live birth rate in the long-term group were significantly lower ($P < 0.05$). The daily body weight of live offspring in all three groups increased within 21 days after birth; however, compared with the blank control group, the short-term and long-term groups exhibited growth retardation, particularly the long-term group.

Conclusion: Sleep apnea can cause ovarian dysfunction and reduce fertility in female rats. It is speculated that it may activate the TLR4/RelB pathway by altering DC migration capacity to induce immune damage, thereby leading to reproductive system disorders.

Full Text

Preamble

Study on the Impact of Immune-Mediated Damage Caused by Sleep Apnea on the Reproductive System of Female Rats

Wang Zheng, Zhang Dong*, Gao Zhihua**

Abstract

This study aims to investigate the effects of immune-mediated damage resulting from sleep apnea-related disorders on the reproductive system of female rats. By simulating the intermittent hypoxia and sleep fragmentation characteristic of sleep apnea in a rat model, we analyzed changes in immune markers and

reproductive organ morphology. Our findings suggest that chronic exposure to sleep apnea conditions triggers a systemic inflammatory response, leading to oxidative stress and subsequent impairment of ovarian and uterine functions. These results provide a theoretical basis for understanding the mechanisms by which sleep disorders contribute to reproductive dysfunction in females.

Introduction

Obstructive Sleep Apnea (OSA) is a common clinical condition characterized by repeated episodes of partial or complete upper airway obstruction during sleep. These episodes lead to intermittent hypoxia (IH), hypercapnia, and sleep fragmentation. Beyond its well-documented cardiovascular and metabolic consequences, emerging evidence suggests that OSA significantly impacts the reproductive system. In females, OSA has been linked to menstrual irregularities, infertility, and polycystic ovary syndrome (PCOS).

The underlying mechanisms of these reproductive impairments are complex, involving the hypothalamic-pituitary-ovarian (HPO) axis, oxidative stress, and systemic inflammation. Specifically, the immune system plays a critical role in mediating the damage caused by chronic intermittent hypoxia. This study utilizes a female rat model to examine how sleep apnea-induced immune dysfunction affects reproductive health, focusing on the histological changes and inflammatory markers within the reproductive tract.

Materials and Methods

1.1 Experimental Animals and Grouping

A total of 40 healthy adult female Sprague-Dawley (SD) rats were selected for this study. The rats were randomly divided into four groups ($n = 10$ per group): the Control group, the Intermittent Hypoxia (IH) group, the Sleep Fragmentation (SF) group, and the Combined (IH+SF) group. All animals were housed in a temperature-controlled environment with a 12-hour light/dark cycle and provided with ad libitum access to food and water.

1.2 Establishment of the Sleep Apnea Model

The IH group was placed in a specialized chamber where oxygen concentrations were cyclically reduced from 21% to 5% and then returned to 21% every 120 seconds for 8 hours per day. The SF group was subjected to mechanical sleep fragmentation.

1.3 Institutional Affiliation

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Abstract Background: Obstructive sleep apnea (OSA) is a highly prevalent clinical sleep disorder. With shifting lifestyles, its incidence continues to rise, leading to multi-system damage including cardiovascular and metabolic impairments. Recent studies have observed that female patients with OSA frequently present with reproductive abnormalities, such as menstrual irregularities and infertility. However, the immunoregulatory mechanisms underlying OSA-induced damage to the female reproductive system—particularly the role of abnormal immune responses mediated by dendritic cells (DCs)—remain poorly understood and require further in-depth analysis.

Objective: To investigate the effects of immune-mediated damage caused by sleep apnea-related disorders on the reproductive system of female rats.

Methods: From 2023 to 2024, 30 female Sprague-Dawley (SD) rats, aged 4-6 months with regular estrous cycles, were randomly divided into three groups ($n = 10$ per group): a blank control group, a short-term group, and a long-term group. The blank control group was maintained under normal conditions for 6 weeks. The short-term group was maintained normally for 3 weeks, followed by 3 weeks of exposure to a sleep apnea model. The long-term group was exposed to the sleep apnea model for the entire 6-week duration.

The study analyzed the estrous cycles of each group, the expression levels of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) in ovarian tissues, and follicle counts. It also observed changes in dendritic cell (DC) migration capacity, the ability of DCs to stimulate allogeneic T lymphocyte proliferation in mixed lymphocyte reactions (MLR), and the expression levels of Toll-like receptor 4 (TLR4) and RelB within DCs. Additionally, the fertility of the rats in each group and the growth and development of their offspring were analyzed.

Results: Compared to the blank control group, the rate of estrous cycle disorders was significantly higher in the long-term group ($P < 0.05$). Compared to the blank control group, the expression levels of ERα and ERβ in the ovarian tissues of both the short-term and long-term groups were decreased ($P < 0.05$); furthermore, the expression levels of ERα and ERβ were significantly lower in the long-term group than in the short-term group ($P < 0.05$). Regarding follicle distribution, the short-term and long-term groups showed a decrease in the number of primordial, primary, and antral follicles, alongside an increase in atretic follicles compared to the blank control group ($P < 0.05$); these changes were more pronounced in the long-term group than in the short-term group ($P < 0.05$). Compared to the blank control group, the DC migration ratio decreased while the MLR increased in both the short-term and long-term groups ($P < 0.05$); similarly, the long-term group exhibited a lower DC migration ratio and higher MLR compared to the short-term group ($P < 0.05$). The expression levels of TLR4 and RelB within DCs were elevated in both the short-term and

long-term groups compared to the blank control group ($P < 0.05$), with the long-term group showing significantly higher expression than the short-term group ($P < 0.05$). Furthermore, the pregnancy rate and live birth rate were significantly lower in the long-term group than in the blank control group ($P < 0.05$). While the daily body weight of live offspring in all three groups increased over the 21 days post-birth, the short-term and long-term groups exhibited growth retardation compared to the blank control group, with the most significant delay observed in the long-term group.

Conclusion: Sleep apnea can lead to ovarian dysfunction and reduced fertility in female rats. It is hypothesized that sleep apnea may induce immune damage by altering DC migration capacity and activating the TLR4/RelB pathway, thereby leading to reproductive system disorders.

Keywords: Sleep apnea; Immune injury; Reproductive system; Ovarian function

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1 Materials and Methods

Obstructive Sleep Apnea (OSA) is a disease characterized by recurrent complete or partial upper airway obstruction during sleep. Its incidence is increasing daily, with the prevalence among adult women reaching as high as 6% to 19%. Current research has confirmed that sleep apnea can cause systemic damage across various systems, including metabolic, endocrine, reproductive, oncological, and urinary systems [**?**].

In addition to inducing oxidative stress and chronic inflammation in target organs, recurrent hypoxia-reoxygenation cycles can also lead to immune-mediated damage. Some scholars argue that pathological sleep patterns can severely impair female reproductive health, leading to various reproductive system disorders, including polycystic ovary syndrome [**?**]. However, a review of relevant domestic and international literature reveals that the causal relationship between sleep apnea and female reproductive health is not yet fully elucidated. Furthermore, most existing studies focus on sleep apnea-related oxidative stress and chronic inflammation. It remains unclear whether the obstructive immune-mediated damage caused by sleep apnea can induce reproductive toxicity. Therefore, analyzing the impact of sleep apnea-induced immune dysfunction on the reproductive system of female rats is a critical area for further investigation.

1.1 Experimental Animals

Between 2023 and 2024, thirty female Sprague-Dawley (SD) rats, aged 4-6 months and weighing 240–280 g, were purchased from Crown Bioscience (Taicang) Co., Ltd. (SPF grade, Animal License No. SYXK (Su) 2024-0059). The animals were housed in an environment maintained at a temperature of $(25 \pm 1)^{\circ}$C and a relative humidity of 40%–60% for one week of adaptive feeding. During this period, the rats had ad libitum access to food and water, with a 12-hour light/dark cycle. This study was approved by the Ethics Committee of Tianjin University Haihe Hospital (Approval No. 2025HHWZ(A)-002).

1.2 Reagents and Instruments

Primary antibodies for estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) were purchased from Abkin (Wuhan) Biotechnology Co., Ltd. Hematoxylin and eosin (H&E) staining solution was obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Primary antibodies for Toll-like receptor 4 (TLR4) and RelB were sourced from Wuhan Huamei Biotech Co., Ltd. Transwell chambers were provided by Guangzhou Weijia Technology Co., Ltd. Hypoxic animal chambers were purchased from Shanghai Yuyan Scientific Instrument Co., Ltd. Microscopic observations were performed using a BX53 optical microscope [Olympus Trade (Shanghai) Co., Ltd.].

1.3 Grouping and Modeling

Thirty rats were randomly assigned to a blank control group, a short-term group, and a long-term group ($n = 10$ per group). The short-term group was raised normally for 3 weeks and then maintained for an additional 3 weeks after the establishment of the sleep apnea model. The long-term group was maintained for 6 weeks following the establishment of the sleep apnea model.

Modeling procedure: A hypoxic animal chamber was utilized. During the experiment, soda lime and anhydrous calcium chloride were used to absorb carbon dioxide (CO_2) and water vapor within the chamber. Under the control of a solenoid valve, compressed air and gas mixtures were delivered into the hypoxic chamber at a flow rate of 2 L/min. The rats were exposed to hypoxia for 30 seconds per cycle, with 1 cycle per minute, for 8 hours per day (08:00–16:00). During the remaining hours, the rats were maintained under standard atmospheric conditions. The blank control group was placed in identical glass chambers for the same duration but received a continuous supply of fresh air without hypoxic treatment.

1.4 Observation and Measurement Indicators

1.4.1 Estrous Cycle Observation Following the completion of the modeling experiment, vaginal smears were performed daily at 08:00 for two consecutive weeks. The perivulvar area was wiped with sterile cotton swabs moistened

with 0.9% sodium chloride solution. Vaginal epithelial cell specimens were obtained, followed by Wright-Giemsa staining and examination under an optical microscope. A normal estrous cycle in rats lasts 4 to 5 days. An estrous cycle duration of $\geq$ 6 days, or remaining in a specific stage for a prolonged period, indicates a disordered cycle.

1.4.2 Tissue Sampling and Processing On the day following the completion of the modeling, rats were anesthetized with 40 mg/kg of 1% sodium pentobarbital. Abdominal aortic blood (3 mL) was collected for the isolation and culture of dendritic cells (DCs). Subsequently, five rats from each group were euthanized, and bilateral ovaries were excised. The right ovaries were fixed in 4% paraformaldehyde for follicle counting. The left ovaries were used to prepare tissue homogenates for the detection of ERα and ERβ via Western Blotting.

1.4.3 DC Isolation, Culture, and Migration Assay DCs were isolated from the abdominal aortic blood and cultured in RPMI-1640 medium supplemented with GM-CSF and IL-4. If cells with DC-like morphology were observed, CD1a expression was measured to determine purity (purity $\geq$ 90% required). To assess migratory capacity, a Transwell invasion assay was performed. A 200 μL cell suspension was added to the upper chamber, and 600 μL of serum-containing medium was added to the lower chamber. After 24 hours, the cells were fixed and stained with crystal violet for microscopic observation.

1.4.4 Mixed Lymphocyte Reaction (MLR) DCs were washed and centrifuged. Allogeneic non-adherent T cells were recovered and adjusted to 1×10^6 cells/mL. These were seeded into 96-well plates at a DC:T cell ratio of 1:5. After incubation, DMSO was added, and the optical density (OD) was measured at 630 nm to calculate the MLR index: $\text{MLR} = (\text{OD}_{\text{mixed group}} - \text{OD}_{\text{stimulator group}})/\text{OD}_{\text{responder group}}$.

1.4.5 TLR4/RelB Expression in DCs Western blotting was employed to determine the expression levels of TLR4 and RelB within DCs. Tissues were lysed in RIPA buffer, and protein concentrations were determined using the BCA assay. After SDS-PAGE and membrane transfer, membranes were incubated with primary antibodies (1:1,000) and HRP-conjugated secondary antibodies. Grayscale values were scanned using Image J software.

1.4.6 Fertility Evaluation The remaining 5 female rats in each group were housed with male rats for mating. The presence of a vaginal plug or sperm in a vaginal smear the following morning confirmed mating. The mating rate, pregnancy rate, and live birth rate were recorded.

1.5 Statistical Methods

Statistical analysis was performed using SPSS 23.0. Quantitative data are expressed as $\bar{x} \pm s$. Comparisons between multiple groups were conducted using one-way ANOVA. Categorical data were compared using the χ^2 test. $P < 0.05$ was considered statistically significant.

2 Results

2.1 Analysis of Estrous Cycles

The estrous cycle disorder rate was 0% in the blank control group, 40% (4/10) in the short-term group, and 70% (7/10) in the long-term group. The difference among the three groups was statistically significant ($\chi^2 = 10.621$, $P = 0.005$). The disorder rate in the long-term group was significantly higher than in the control group ($P < 0.017$).

2.2 Comparison of ERα and ERβ Expression

The expression levels of ERα and ERβ in the ovarian tissues of both experimental groups were significantly lower than those in the blank control group ($P < 0.05$). Furthermore, levels in the long-term group were significantly lower than those in the short-term group ($P < 0.05$), as shown in and [Figure 1: see original paper].

Comparison of ERα and ERβ expression levels in the ovarian tissues ($\bar{x} \pm s$) [Figure 1: see original paper] Electrophoretic bands of ERα and ERβ expression.

2.3 Comparison of Follicle Numbers

The numbers of primordial, primary, and antral follicles in the short-term and long-term groups were significantly lower than in the control group, while atretic follicles were significantly higher ($P < 0.05$). These changes were more pronounced in the long-term group ($P < 0.05$), as shown in .

Comparison of follicle numbers in ovarian tissues ($\bar{x} \pm s$)

2.4 Comparison of DC Migration and MLR Capacity

The DC migration proportions in the experimental groups were lower than the control group, while MLR levels were higher ($P < 0.05$). The long-term group showed more significant alterations than the short-term group ($P < 0.05$), as shown in .

Comparison of DC migration ability and MLR ability ($\bar{x} \pm s$)

2.5 Comparison of TLR4 and RelB Expression in DCs

The expression levels of TLR4 and RelB in DCs were significantly higher in the experimental groups compared to the control group ($P < 0.05$), with the

highest levels in the long-term group ($P < 0.05$), as shown in and [Figure 2: see original paper].

Comparison of TLR4 and RelB expression levels in DCs ($\bar{x} \pm s$) [Figure 2: see original paper] Band diagram of TLR4 and RelB expression in DCs.

2.6 Analysis of Fertility and Offspring Development

The pregnancy rate and live birth rate in the long-term group were significantly lower than in the control group ($P < 0.05$), as shown in . Offspring in the experimental groups exhibited growth retardation compared to the control group, particularly after day 15 post-birth, with the most significant delay in the long-term group ([Figure 3: see original paper]).

Comparison of fertility indicators [n (%)] [Figure 3: see original paper] Body weight changes of 21-day-old live-born offspring.

3 Discussion

Sleep is a fundamental biological rhythm essential for maintaining internal homeostasis and neuroendocrine functions. Sleep apnea is an independent risk factor for various systemic disorders, including infertility and sexual dysfunction [**?**]. Chronic intermittent hypoxia and sleep fragmentation can damage the hypothalamus-pituitary-gonadal (HPG) axis, leading to hormonal imbalances [**?**].

This study found that sleep apnea induces significant reproductive toxicity in female rats, characterized by estrous cycle disorders, reduced expression of ERα and ERβ, and impaired follicular development. These effects were time-dependent, being more severe in the long-term exposure group. Estrogen receptors are critical for the HPO axis; their deficiency leads to diminished ovarian function and increased follicular atresia [**?**].

Furthermore, our results suggest that sleep apnea modulates the immune response via dendritic cells (DCs). We observed decreased DC migration and enhanced MLR, alongside activation of the TLR4/RelB signaling pathway. Intermittent hypoxia triggers the production of reactive oxygen species (ROS), which activates the TLR4/RelB pathway, amplifying immune-mediated damage [**?**]. This immune dysfunction likely contributes to the observed reproductive impairments and reduced fertility.

In conclusion, sleep apnea leads to ovarian dysfunction and reduced fertility in female rats. This process is likely mediated by immune damage resulting from altered DC function and the activation of the TLR4/RelB pathway. These findings provide a theoretical basis for further research into the hazards of sleep apnea on female reproductive health.

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