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Effect of Sleep Deprivation on the Male Reproductive System in Rats

Ji Ho Choi,[1] Seung Hoon Lee,[2] Jae Hyun Bae,[3] Ji Sung Shim,[3] Hong Seok Park,[3] Young Sik Kim,[4] and Chol Shin[5]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Soonchunhyang University College of Medicine, Bucheon Hospital, Bucheon, Korea; [2]Department of Otorhinolaryngology-Head and Neck Surgery, Korea University College of Medicine, Seoul, Korea; [3]Department of Urology, Korea University College of Medicine, Seoul, Korea; [4]Department of Pathology, Korea University College of Medicine, Seoul, Korea; [5]Department of Respiratory Internal Medicine, Korea University College of Medicine, Seoul, Korea

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Address for Correspondence:
Chol Shin, MD
Department of Respiratory Internal Medicine, Korea University College of Medicine, Ansan Hospital, 123 Jeokgeum-ro, Danwon-gu, Ansan 15355, Korea
E-mail: chol-shin@hanmail.net

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There has been no study reporting on the influence of sleep deprivation on the male reproductive system including sperm quality. In this study, we hypothesized that sleep deprivation could lead to adverse effect on the male reproductive system. The rats were divided into three groups: 1) control (home-cage, n = 10); 2) SD4 (sleep deprivation for 4 days, n = 10); and 3) SD7 (sleep deprivation for 7 days, n = 10). Sleep deprivation was performed by a modified multiple platform method. Sperm quality (sperm motion parameters and counts), hormone levels (corticosterone and testosterone), and the histopathology of testis were evaluated and compared between the three groups. A statistically significant reduction ($P = 0.018$) was observed in sperm motility in the SD7 group compared to those of the control group. However, there were no significant differences in other sperm motion parameters, or in sperm counts of the testis and cauda epididymis between three groups. Compared with the control group, the SD4 ($P = 0.033$) and SD7 ($P = 0.002$) groups exhibited significant increases of corticosterone levels, but significant decreases of testosterone levels were found in the SD4 ($P = 0.001$) and SD7 ($P < 0.001$) groups. Seminiferous tubular atrophy and/or spermatid retention was partially observed in the SD4 and SD7 groups, compared with the normal histopathology of the control group. Sleep deprivation may have an adverse effect on the male reproductive system in rats.

Keywords: Sleep Deprivation; Sperm Motility; Sperm Count; Corticosterone; Testosterone; Histopathology

INTRODUCTION

According to a recent paper on the fertility rate in the United States over the last four decades, the general fertility rate, number of births to women aged 15-44 years, was about 45% lower in 2002 than that in 1960 (1). Coinciding, a remarkable decline in fertility rates has been shown in other industrialized areas (2). It is believed that the decline is mainly caused by the changing social structures in the industrialized society, as well as changes in contraceptive practices. There is an ongoing debate as to whether a worldwide decline in semen quality is exists (3-8), and recently, it has been proposed that low semen quality may be related to the decline in fertility rates (9).

Although sleep comprises about one-third of one's lifetime, we do not fully understand or recognize its importance. According to the National Sleep Foundation poll (10), a significant proportion of respondents (39%) report getting less than 7 hours of sleep on weeknights. Furthermore, the literature shows that self-reported sleep durations decreased by about 1.5-2 hours over the past several decades (10,11). The adequate amount of sleep (duration in hours) for adult is a controversial topic, but generally, it is suggested that approximately 8 hours of night time sleep is adequate, and perhaps, optimal for normal bodily functions (12-14).

Sleep is generally defined as a rapid and reversible state of behavioral immobility with greatly reduced sensory responsiveness, and is associated with homeostatic regulation of the autonomic, neuroendocrine and immune systems (15). The specific function(s) of sleep remain unclear, but sleep does have fundamental and important physiologic role in survival. Sleep deprivation can be defined as the partial or near-complete removal of sleep in an organism that produces various harmful health problems (16). Common sleep disorders including behaviorally induced insufficient sleep syndrome, obstructive sleep apnea syndrome, and insomnia, are related to sleep deprivation and/or fragmentation (17).

In human and animal models, accumulating data suggest that sleep deprivation or loss has been associated with numerous adverse physiological consequences such as cardiovascular, immune, metabolic and endocrine disturbances (18-22).

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Short sleep duration in humans is related to increased incidence of mortality; sustained sleep deprivation in experimental animal models leads to death (22,23). Hence, sleep deprivation, as a harmful stress, in both humans and animals, may adversely affect organism's well-being by negatively impacting the normal function of vital organs the brain, heart, liver, kidneys and sex organs, such as testis and epididymis.

However, to our knowledge, there has been no study reporting on the influence of sleep deprivation on the male reproductive system including sperm quality, which may be related to fertility rate in animal models and humans. Therefore, we hypothesized that sleep deprivation could lead to adverse effect on the male reproductive system. To test this hypothesis, we conducted a prospective study to evaluate 1) sperm quality, 2) hormone levels, and 3) histopathology of testis associated with the male reproductive system in sleep-deprived rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (aged 10 weeks, weighing 300-400 g) were obtained from the Orient Bio Co. Ltd. (Seongnam, Korea) and used for the study after 1 week of a quarantine and acclimation period. The animals were housed in a room maintained at a temperature of 23°C ± 3°C and a relative humidity of 50% ± 10% with artificial lighting from 08:00 to 20:00 (12:12 hours light-dark cycle) and with 13-18 air changes per hour. Animals were housed in a stainless steel, wire mesh cage during the acclimation period, and only healthy animals were assigned to the study. Pelleted foods for experimental animals were purchased, from the PMI Nutritional International Inc. (Richmond, IN, USA), gamma ray irradiated and given ad libitum. UV-irradiated municipal tap water was given ad libitum. The experiments were approved by the Institutional Animal Care and Use Committee of the Korea University.

Sleep deprivation

Sleep deprivation was performed by a modified multiple platform method, one of the most common sleep deprivation methods for rats (24). Experimental rats were placed in a custom-made acryl tank (123 × 44 × 44 cm) containing 14 round platforms of 6.5-cm-diameter. The tank was filled with water to about 1 cm below the platform surface. The rats can move around by leaping from one platform to another in a tank. When the rats enter rapid eye movement (REM) sleep, the loss of muscle tone occurs and it causes the rats to make contact with the water, thereby, the rats are abruptly awakened. This technique has been known to not only eliminate REM sleep, but to fragment slow wave sleep. The rats were accustomed to the novel environment by placing them on the platforms for 1 hour, for consecutive 3 days, before sleep deprivation was performed.

Study design

After quarantine and acclimation period, thirty rats, with adequate weight gain and without clinical symptoms, were randomly distributed into three experimental groups. There were no differences in statistical significance and standard deviation between groups in body weight before the experiments. There were three experimental groups: 1) the control (home-cage, n = 10); 2) SD4 (sleep deprivation for 4 days, n = 10); and 3) SD7 (sleep deprivation for 7 days, n = 10). The SD groups, SD4 and SD7, were subjected to sleep deprivation for 4 days and 7 days, respectively. During sleep deprivation, the control group was maintained in separate cages in the same room as the experimental groups. Pelleted foods and water were also given ad libitum.

Sperm motion parameters

After the rats were euthanized with CO_2 asphyxiation, both testes and cauda epididymides were removed. To measure motile parameters of sperm, bovine serum albumin (Sigma, St. Louis, MO, USA) was dissolved to be 0.5% in CO_2-independent medium (Gibco, Grand Island, NY, USA), with media pH was adjusted to approximate 7.4, and then media were kept in a water bath at 37°C. Five mL of media were placed in a petri dish. To suspend sperm, the distal portion of the left cauda epididymis was punctured with a 23-gauge needle in the medium, and then, sperm were exuded in the medium by gentle squeezing of the proximal portion. Sperm motion analysis was conducted at 5-10 minutes after preparation using the HTM-TOX IVOS sperm analysis system (Version 12.3, Hamilton Thorne Bioscience, Beverly, MA, USA).

Setting values of HTM-TOX IVOS sperm analysis system

Frames per second: 60 Hz; Number of frames: 30; Minimum contrast for motile cell detection: 60; Minimum cell size of motile cell detection: 2 pixels; Static intensity gates: 0.28-1.84; Static size gates: 0.61-3.85; Static elongation gates: 3-27; VAP cutoff for non-motile cells: 20 μm/sec; VSL cutoff for non-motile cells: 30 μm/sec.

Sperm motion parameters

1) Motility: percentage of motile spermatozoa (%).
2) Path velocity (VAP): the average velocity of the smoothed cell path (μm/sec).
3) Straight line velocity (VSL): the average velocity measured in a straight line from beginning to end of the track (μm/sec).
4) Curvilinear velocity (VCL): the average velocity measured over the actual point to point track followed by the cell (μm/sec).
5) The amplitude of the lateral head displacement (ALH): the mean widths of the head oscillation as the sperm cells swim (μm).

6) The beat cross-frequency (BCF): frequency of sperm head crossing the average path in either direction (Hz).
7) Straightness (STR): average value of the ratio VSL/VAP (%).
8) Linearity (LIN): average value of the ratio VSL/VCL (%).

Sperm counts

The tunica albuginea of obtained left testis was peeled off and testicular tissue was homogenized with 12 mL distilled water. Prepared sperm suspension was put into the hemocytometer (Neubauer, Germany), and after the samples were left for approximately 5 minutes for sperm heads to stabilize, the number of testicular sperm heads was counted at a magnification × 200 using a light microscope.

To measure sperm head counts in the cauda epididymis, left cauda epididymis was minced with a fine scissor and then placed in a 50 mL plastic tube containing 5 mL distilled water. After observation of sperm motion parameter, remnant sperm suspension of the cauda epididymis was also placed into the tube. This suspension was homogenized for > 1 minute and then sonicated at 4°C for 3 minutes. Sperm head counts of the cauda epididymis were examined according to the same procedures for the testis.

Blood sampling for hormone measurements

After the rats were euthanized with CO_2 asphyxiation, blood was collected from the cauda vena cava using sterile tubes containing EDTA and was centrifuged for 15 minutes at 3,000 rpm. Serum samples were stored at -80°C until assayed for testosterone and corticosterone. Corticosterone levels were measured by an enzyme-linked immunosorbent assay (ELISA) method, using a commercial kit (Assay Designs, New York, NY, USA). Testosterone levels were measured by an enzyme-linked immunosorbent assay (ELISA) method, using a commercial kit (Assay Designs).

Histopathological evaluation

The other (right) testis was fixed in Bouin's solution, dehydrated in ethyl alcohol, cleaned in xylene, and embedded in paraffin wax. The tissue blocks were cut 5 μm in thickness, stained with hematoxylin & eosin (H&E), periodic acid Schiff (PAS) stains, and examined under a light microscope.

Statistics

Continuous variables in the data were summarized as mean ± standard deviation (SD) and categorical variables were represented as frequencies (percentage). Statistical analysis of the data was performed using the SPSS (Version 10.0, Chicago, IL, USA) for Windows software. A one-way ANOVA, followed by Scheffe test, was used to assess differences of sperm motion parameters and counts, and corticosterone and testosterone levels between the control, SD4, and SD7 groups. A P value of < 0.05 was considered to be statistically significant.

Ethics statement

All animal care and experimental procedures were carried out with approval by the Korea University Hospital Institutional Animal Care and Use Committee according to the animal experimentation guidelines of the Korea University Ansan Hospital Animal Laboratory (KUIACUC-2009-87).

RESULTS

Sperm motion parameters

Fig. 1 shows the motility of sperm between the control, SD4, and SD7 groups. There were significant differences ($F_{2,27}$ = 4.694, P = 0.018) in the motility of sperm (control, 85.5% ± 3.6%; SD4, 80.7% ± 4.4%, and SD7: 76.8% ± 9.4%) between the groups. Compared to the control group, a statistically significant reduction (P = 0.018) was observed in sperm motility of the SD7 group. However, no significant differences were found in sperm motility between the control and SD4 groups.

There were no significant differences in any other motion parameters of sperm such as VAP ($F_{2,27}$ = 0.256, P = 0.776); VSL ($F_{2,27}$ = 0.040, P = 0.961); VCL ($F_{2,27}$ = 0.095, P = 0.910); ALH ($F_{2,27}$ = 0.515, P = 0.603); BCF ($F_{2,27}$ = 2.192, P = 0.131); STR ($F_{2,27}$ = 0.342, P = 0.714); and LIN ($F_{2,27}$ = 0.307, P = 0.738) among the control, SD4 and SD7 groups (Table 1).

Sperm counts

There were no significant differences in sperm counts of testis ($F_{2,27}$ = 0.510, P = 0.606) and cauda epididymis ($F_{2,27}$ = 0.566, P = 0.574) between the control, SD4, and SD7 groups (Table 2).

Corticosterone hormone levels

Fig. 2 displays the concentration of corticosterone between the

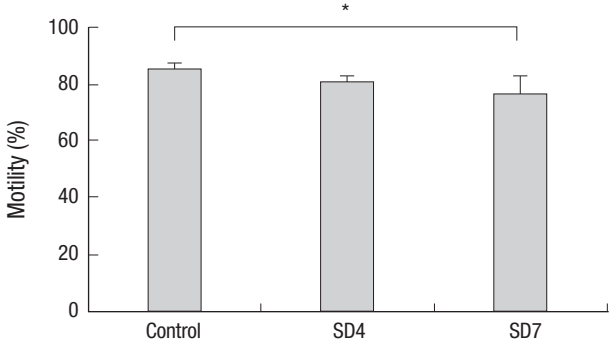

Fig. 1. Comparison of sperm motility between the control, SD4, and SD7 groups. There are significant differences ($F_{2,27}$ = 4.694, P = 0.018) in the motility of sperm (control: 85.5% ± 3.6%; SD4: 80.7% ± 4.4%; and SD7: 76.8% ± 9.4%) between the control, SD4, and SD7 groups. Compared to those of the control group, a statistically significant reduction (P = 0.018) was found in sperm motility of the SD7 group. Data are presented as means ± SD; One-way ANOVA followed by Scheffe test.
SD4, sleep deprivation for 4 days; SD7, sleep deprivation for 7 days.
*P < 0.05, significant difference between the control, SD4, and SD7 groups.

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Table 1. Comparison of sperm motion parameters between the control, SD4, and SD7 groups

Parameters	Control	SD4	SD7
Motility, %	85.5 ± 3.6*	80.5 ± 4.4*,†	76.8 ± 9.4†
VAP, μm/sec	132.8 ± 8.1	134.4 ± 16.5	137.1 ± 14.9
VSL, μm/sec	89.7 ± 8.4	91.1 ± 12.1	90.7 ± 13.3
VCL, μm/sec	288.7 ± 26.0	283.1 ± 34.5	283.1 ± 38.4
ALH, μm	16.2 ± 1.8	17.3 ± 3.5	16.7 ± 2.1
BCF, Hz	25.8 ± 3.3	23.3 ± 2.9	23.5 ± 2.5
STR, %	69.3 ± 4.5	69.6 ± 4.8	67.7 ± 7.0
LIN, %	32.3 ± 2.2	33.2 ± 2.9	33.4 ± 4.5

Data are presented as means ± SD; One-way ANOVA test followed by Scheffe test.
SD4, sleep deprivation for 4 days; SD7, sleep deprivation for 7 days; VAP, path velocity; VSL, straight line velocity; VCL, curvilinear velocity; ALH, amplitude of the lateral head displacement; BCF, beat cross-frequency; STR, Straightness; LIN, Linearity.
*,†Different mark indicates significant difference at level of 0.05.

Table 2. Comparison of sperm counts between the control, SD4, and SD7 groups

Sperm counts ($\times 10^6$)	Control	SD4	SD7
Testis	249.8 ± 51.1	249.3 ± 31.5	233.9 ± 34.7
Cauda epididymis	123.4 ± 26.5	113.1 ± 23.4	111.6 ± 30.8

Data are presented as means ± SD; One-way ANOVA test followed by Scheffe test; No significant group difference at level of 0.05.
SD4, sleep deprivation for 4 days; SD7, sleep deprivation for 7 days.

control, SD4, and SD7 groups. There were significant differences ($F_{2,27} = 7.870$, $P = 0.002$) in the concentration of corticosterone between the control and SD groups. Compared to those of the control group, significant increases of corticosterone levels were observed in the SD4 ($P = 0.033$) and SD7 ($P = 0.002$) groups. However, there were no significant differences in levels of corticosterone between the SD4 and SD7 groups.

Testosterone levels

Fig. 3 presents the concentration of testosterone between the control, SD4, and SD7 groups. There were significant differences ($F_{2,26} = 15.438$, $P < 0.001$) in the concentration of testosterone between the groups. Compared to the control group, there were significant decreases of testosterone levels in the SD4 ($P = 0.001$) and SD7 ($P < 0.001$) groups. However, no differences were found in levels of testosterone between the SD4 and SD7 groups.

Histopathology evaluation

Fig. 4 shows histopathological findings of the testis in the control, SD4, and SD7 groups. Abnormal morphology of seminiferous tubules was seen in the SD4 and SD7 groups, while normal morphology was observed in the control group. In the SD4 group, spermatid retention was found in the seminiferous tubules. Furthermore, in the SD7 group, spermatid retention and/or tubular atrophy and diffuse germ cell disorganization were observed in the seminiferous tubules (Fig. 4D).

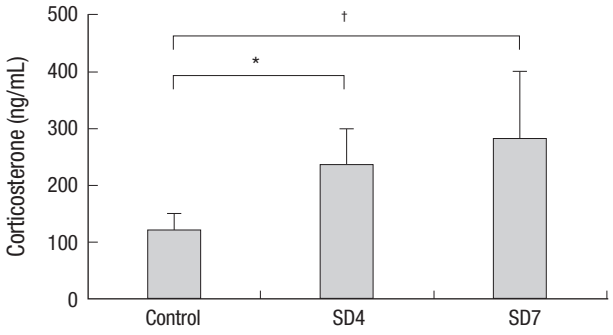

Fig. 2. Comparison of concentration of corticosterone between the control, SD4, and SD7 groups. There are significant differences ($F_{2,27} = 7.870$, $P = 0.002$) in the concentration of corticosterone between the control, SD4, and SD7 groups. Compared to those of the control group, significant increases in corticosterone levels were found in the SD4 ($P = 0.033$) and SD7 ($P = 0.002$) groups. Data are presented as means ± SD; One-way ANOVA followed by Scheffe test.
SD4, sleep deprivation for 4 days; SD7, sleep deprivation for 7 days.
*$P < 0.05$, †$P < 0.01$, significant difference among the control, SD4, and SD7 groups.

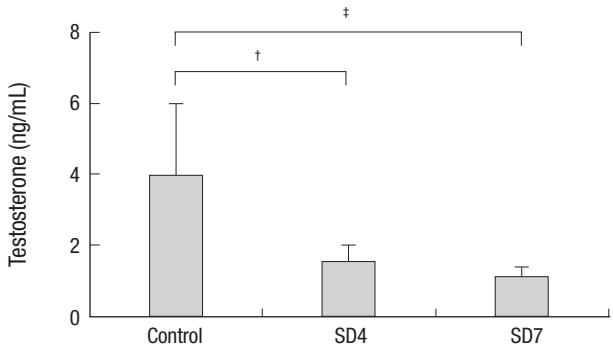

Fig. 3. Comparison of concentration of testosterone between the control, SD4, and SD7 groups. There are significant differences ($F_{2,26} = 15.438$, $P < 0.001$) in the concentration of testosterone between the control, SD4, and SD7 groups. Compared to those of the control group, there were significant decreases in testosterone levels in the SD4 ($P = 0.001$) and SD7 ($P < 0.001$) groups. Data are presented as means ± SD; One-way ANOVA followed by Scheffe test.
SD4, sleep deprivation for 4 days; SD7, sleep deprivation for 7 days.
*$P < 0.01$, †$P < 0.001$, significant difference between the control, SD4, and SD7 groups.

DISCUSSION

To determine whether sleep deprivation has an effect on the male reproductive system, the present study attempted to evaluate sperm quality, hormone levels, and histopathology of testis in sleep-deprived male rats. Results of the current study indicate that sleep deprivation may influence the male reproductive system in rats. To our knowledge, this is the first study that identifies the detrimental effects of sleep deprivation on the male reproductive system, sperm and testis, in rats.

In this study, we examined sperm motion parameters and counts to investigate sperm quality in sleep-deprived male rats. A statistically significant reduction was found in sperm motility of the SD7 group compared to those of the control group. How-

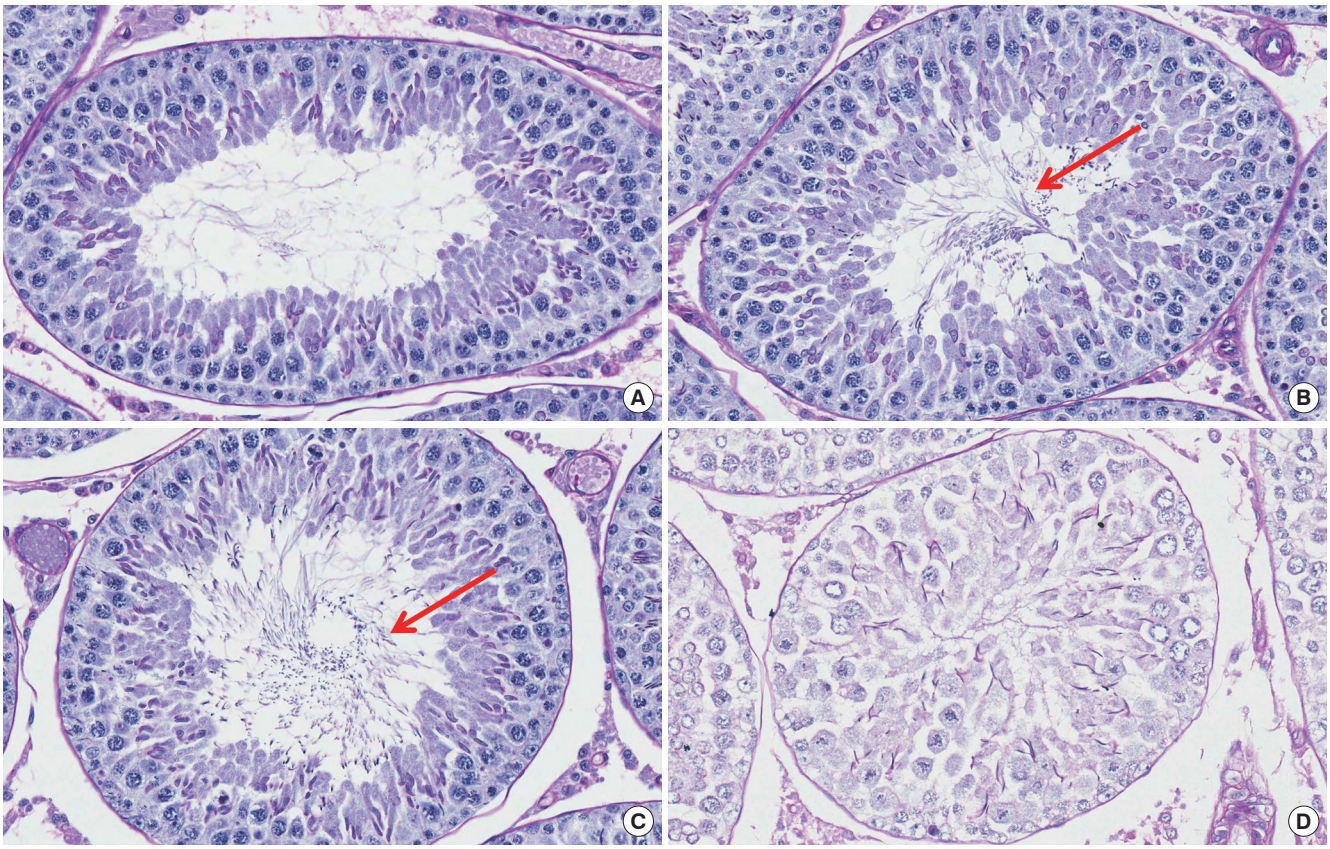

Fig. 4. Histopathology of the testis in the control, SD4, and SD7 groups. Normal morphology of seminiferous tubules is seen in the control group. Abnormal morphology of seminiferous tubules is seen in the SD4 and SD7 groups. (**A**) The view of the control group: Normal finding of seminiferous tubules (Stages IX-XI of spermatogenesis). (**B**) The view of the SD4 group: Seminiferous tubules with spermatid retention (red arrow) (Stages IX-XI of spermatogenesis). (**C**) The view of the SD7 group: Seminiferous tubules with spermatid retention (red arrow) (Stages IX-XI of spermatogenesis). (**D**) The view of the SD7 group: Tubular cell depletion and germ cell disorganization (Stages XIII-XIV of spermatogenesis). PAS stain, magnification, × 400.

ever, there were no significant differences in any other parameters between the control and SD groups. There were insignificant decreases in sperm counts of testis and cauda epididymis between the control and SD groups. In the present study, the sperm motility in one male of the SD7 group decreased to a level of 53%, while the sperm motility of all subjects in the control group was within the normal range. The precise mechanism responsible for the difference in sperm motility between the control and SD7 groups is unknown. However, as mentioned above, sleep deprivation includes stress as an intrinsic part and induces many injurious health problems with endocrinologic, immunologic and metabolic consequences. Therefore, it is speculated that unknown factors and affected sleep duration, associated with sleep deprivation, may contribute to differences in sperm motility. Additional research linking sleep deprivation with sperm motility is necessary, and future work will focus on the mechanism of, or reason for, this result (27). In the current study, we used the computer-assisted sperm analysis (CASA) systems to evaluate sperm motion parameters in male rats. CASA systems have several advantages, including the potential for standardization of sperm analysis procedures, the ability to obtain quantitative objective data and to assess various parameters that cannot be measured manually, such as; average path velocity, straight line velocity, curvilinear velocity, lateral head displacement, flagellar beat frequency, straightness and linearity (25-27). The disadvantages of CASA devices, however, include high-price, limited clinical utility of most parameters, and the possibility that results can be affected by various interfering factors (27).

Corticosterone concentrations in the SD groups were significantly increased, whereas testosterone concentrations in the SD groups were significantly decreased compared with the control group. These results are good in agreement with those of numerous studies that measure blood steroid hormone levels in sleep deprived animals (28-30). The exact mechanism underlying for alterations and/or interactions of corticosterone and testosterone levels associated with sleep deprivation is not fully understood. However, several related pathways have been revealed. The increase in corticosteroid levels by stressful stimuli may inhibit the hypothalamus-pituitary-gonadal (HPG) axis leading to decreased testosterone secretion (31-33). Additionally, declines in testosterone production may be associated with

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activation of the hypothalamus-pituitary-adrenal (HPA) axis inducing elevations in corticosteroid (34). Consequently, it is presumed that during sleep deprivation, with some degree of stress, testosterone levels decrease via inhibition of the HPG axis caused by elevated corticosteroid. Corticosterone levels increase via activation of the HPA axis. Under regulation by the hypothalamus, the pituitary gland produces pituitary gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates the Leydig cells, which have surface receptors for LH; the excited Leydig cells produce and secrete testosterone. Finally, released testosterone controls spermatogenesis, probably by influencing Sertoli cells in the seminiferous tubules. It is well known that testosterone has important androgenic roles in the body in androgenic including promotion of spermatogenesis and development of spermatogenic tissues (25,35). However, if corticosterone levels become elevated by stress, such as in sleep deprivation, production of testosterone in Leydig cells declines and apoptosis of Leydig cells is induced (36). In this study, corticosterone concentrations in the SD7 group increased about 3-fold, while testosterone concentrations were decreased about 1/4-fold compared to those in the control group.

Detrimental effects of sleep deprivation on male reproductive function, including a decrease in sperm motility, were further confirmed by abnormal testicular histopathological findings, as well as hormonal changes. There were no abnormal morphologies of seminiferous tubules in the control group. On the other hand, spermatid retention in the seminiferous tubules was observed in two subjects (20%) of the SD4 group, and in five subjects (50%) of the SD7 group. In addition, atrophy of seminiferous tubules was found in three subjects (30%) of the SD7 group. These results indicate that a prolonged excess period of corticosterone and a suppression period of testosterone, related to the duration of sleep deprivation, may lead to more severe harmful effects on histopathology of testis in male rats (37). In view of the testicular histopathological results of the SD4 and SD7 groups, testicular tissue may begin to be affected by sleep deprivation at 4 days.

Spermatoid retention means delayed spermiation. The pathogenesis is functional disturbance in the process of spermiation, which may be due to abnormalities in the Sertoli cell or the mature spermatids or due to reduction in testosterone levels. Spermatid retention is a subtle but important change because it is frequently associated with abnormalities in sperm parameters and may also be associated with decreased fertility (38,39).

Tubular degeneration and its sequel, tubular atrophy, are common manifestations of toxicologic injury to the testis (40), encompassing effects mediated through Sertoli cell injury, hypoxia, hormonal disruption, inflammation or vascular effects. It is an end-stage lesion where there are no germ cells left within a tubule. In our results, there were significant decreases of testosterone levels in the SD group. These hormonal disruptions were the common cause of spermatoid retention and tubular atrophy which result in decreased fertility. Further studies will be required to assess whether sleep deprivation in male rats has an effect on mating, gestation and implantation, and whether sleep deprivation, or insufficient sleep, may influence the male reproductive system, sperm and testis, in humans.

In conclusion, the results of this study provide more evidence that sleep deprivation may influence sperm quality, hormone levels, and histopathology of testis, associated with the male reproductive system in rats. Since shortened sleep duration may adversely affect the male reproductive system, sleep deprivation should be considered as a possible contributing factor in any decline of male fertility rates.

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DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conception and design of the study: Choi JH, Shin C. Acquisition of data: Choi JH, Shim JS. Statistical analysis: Choi JH, Lee SH. First draft of manuscript: Choi JH. Revision: Choi JH, Lee SH, Shim JS, Park HS, Kim YS. Critical review of the manuscript: Choi JH, Bae JH. Manuscript approval: all authors.

ORCID

Ji Ho Choi http://orcid.org/0000-0002-5194-930X
Jae Hyun Bae http://orcid.org/0000-0001-9862-3545
Ji Sung Shim http://orcid.org/0000-0002-6745-1776
Chol Shin http://orcid.org/0000-0002-2928-8576

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