REPRODUCTIVE PARAMETERS OF MALE ALBINO RATS AFTER INDUCTION IN SLEEP DEPRIVATION MODELS

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Abstract

Paradoxical sleep deprivation (PSD) and total sleep deprivation (TSD) cause disrupt of male infertility but sleep recovery (SR) can improve male reproduction function that connected with occupational works health. The aim of this study is to determine the difference of reproduction parameters in male albinorats after exposed by various sleep deprivation models. This research was experimental post-test only with control group design. Rats were divided into 5 groups (6 animals each group) : negative control, PSD (II), TSD (III), PSD with SR, TSD with SR. The study results showed that average of spermatogenic group IV (8.35 ± 0.06) and V (8.27 ± 0.27) had higher scores. group IV had the highest number of Leydig cell (5,91 ± 1,43), group I had the highest rates (40,02 ± 2,04) number of Sertoli cell and there were no significant difference average of epithelial diameter (p=0,598) and height (p=0,895). There were significant difference of spermatogenic score post-SR, number of Sertoli and Leydig cells, but no significant difference of epithelial diameter and height of seminiferous tubule after exposed by various sleep deprivation stress model. Sleep recovery in occupational work can repair the histological parameter of reproduction.

Key word : male albino rats, reproduction parameters, sleep deprivation models

INTRODUCTION

The modern society tends to have a lifestyle with a high workload and increased activity that is related with the advances of technology and communications which cause significant sleep deprivation (SD). The reduced amount of sleep can induce stress condition, affect sexual function, and potentially lower the fertility.[1] More than 30% of men and women of reproduction age (30- 64 years) only slept for less than six hours of each day.[2] The stress of sleep deprivation interfere with the reproduction system by activating hypothalamus pitutitary adrenal (HPA) axis and the production of glucocorticoid which resulted in reduced secretion of gonadotropin releasing hormone (GnRH) in the hypothalamus and then reduces the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH).[3] Decreased levels of FSH impact on dysfunction and obstruction in Sertoli cell proliferation because these cells have receptors for FSH. The decreasing of LH secretion also decreases testosterone levels so that the production of nutrients for spermatogenesis will be disrupted.[4] Increased secretion of glucocorticoids also induce oxidative stress due to an increased production of reactive oxygen species (ROS). Excessive ROS production can cause damage to the Sertoli cells.[5]

Stress from lack of sleep (sleep deprivation) in humans can be analogous to various models of stress that is paradoxical sleep deprivation (PSD) and total sleep deprivation (TSD). The impact of sleep deprivation can be corrected with recovery sleep which is sleep time given after sleep deprivation.[6] Sleep recovery can restore the antioxidant activity in inhibiting oxidative stress and restore reproduction function as before.[7] The study comparing the effects of various models of sleep deprivation stress on parameter of reproduction histologist has never been done. Therefore, researchers interested in studying differences in the number of spermatogenic cells, Leydig celss and Sertoli cells in male albino rats (Rattus norvegicus) after exposed by various sleep deprivation stress model.

RESEARCH METHODS

The experimental research used posttest only with control group design. This study involved male albino rats (Rattus norvegicus) Wistar's heredity with inclusion

and exclusion criteria that derived from the Experimental Animals Laboratory of Faculty of Medicine, Unsoed. The subject were randomly allocated using completely randomized design (CRD) into five groups: group I, II, III, IV, and V with each group containing six rats. Acclimatization is performed for seven days and given the feed Comfeed AD II and mineral water AQUA® as the drink that were provided ad libitum.

Treatment was given to each group as follows:[8]

1. Healthy control group (K I): groups of rats which were not treated with various sleep deprivation stress model
2. Treatment group I (K II): groups of paradoxical sleep deprivation for 96 hours with 18 hours of sleep deprivation at 04:00 to 22:00 pm and 6 hours of rest at 10 p.m. to 4:00 pm conducted continuously using a modified multiple platform method (MMPM) model equipped by muscle atonia that provide a surprising effect automatically every 10 minutes in experimental animals, so that when the animal entered a phase of sleep, the animal fell into the water and woke again.
3. Treatment group II (K III): a group of total sleep deprivation for 96 hours, sleep deprivation was conducted at 4:00 to 4:00 pm without breaks using MMPM models.
4. Treatment group III (K IV): a group of paradoxical sleep deprivation for 96 hours with 18 hours of sleep deprivation at 4:00 to 22:00 pm and 6 hour break at 22:00 to 4:00 pm conducted continuously using the model of MMPM continued recovery sleep for 96 hours fully at 04:00 to 4:00 pm.
5. Treatment group IV (K V): a group of total sleep deprivation for 96 hours of sleep deprivation at 4:00 to 4:00 pm using the model of MMPM continued recovery sleep for 96 hours at 4:00 to 4:00 pm

Animal's weight is measured before sleep deprivation treatment, after sleep deprivation, and after recovery sleep. At the end of the treatment, we carried out termination of experimental animals and then performed organ harvesting for the right testicle to be observed histologically by made preparations stained with hematoxylin-eosin (HE) in the Research Laboratory of Faculty of Medicine of Jenderal Soedirman University. Spermatogenic cells, Leydig and Sertoli cells counted with 400x magnification in 50 fields of view of each preparation, followed calculating their mean of each treatment group. Observations were carried out at the Laboratory of Histology Faculty of Medicine, Jenderal Soedirman University with the help of inter-observer using light microscope Motic® B2-series equipped with Optilab® and software Raster Image 3.

The body weight average of rats was tested for normality test using Shapiro Wilk test. Data was analyzed using the Wilcoxon test. The mean of spermatogenic cells, Leydig and Sertoli cells were tested for normality test using Shapiro Wilk test and for homogeneity variance test using Levene's test. Bland-Altman test was used to test the suitability of the researchers' data to inter-observer 1 and inter-observer 2. Hypothesis were tested using the comparative One Way ANOVA to determine the significance of differences between the mean number of cells and continued Post-Hoc Tukey to determine which groups have significant differences at $p<0.05$. This study was approved by the Research Ethics Committee of the Faculty of Medicine Jenderal Soedirman University numbered 162/KEPK/IX/2016 dated October 5, 2016.

RESULTS

Table 1 shows that the group I did not change in body weight during the study. Average of body weight of subjects in group II, III, IV, and V decreased after PSD and TSD exposure and recovery sleep after the treatment, on the other hand, average of body weight in groups IV and V were increased. Table 2, showed the average of Sertoli cells number. There is significant difference between treatment groups ($p = 0.000$). Tukey HSD post hoc test showed that group III was significantly different with all treatment groups.

Mann-Whitney post hoc test test showed that the mean of spermatogenic scores significantly different ($p <0.05$) between the healthy control group I with all treatment groups, and the mean of group III had the most significant decrease. The significant differences available in group III

with group IV and V. The number of Sertoli cells, the Post-Hoc Tukey test showed significant differences (p <0.05) in group I-II I-III, II-III, II-IV, II -V, III-IV, and III. In Sertoli cells, one way ANOVA hypothesis testing, it was found that the value (p = 0.000), which means there are significant differences between the treatment groups and Tukey HSD post hoc test showed that the group III was significantly different with group I (p=0.000), group II (p=0.037), group IV (p=0.000) and group V (p=0.000).

Histologically, Leydig cells (Figure 1) showed that group III (TSD without sleep recovery) had least average of number of Leydig cells than other groups, while group IV (PSD with sleep recovery) had the greatest of Leydig cells than other groups. Sleep recovery proved to be influential in the improvement of Leydig cell number, it can be observed by looking at the average group IV (PSD with sleep recovery) and V (TSD with sleep recovery).

Table 1. The Mean Weight Of Experimental Animals

Groups (n=6)	Mean weight (grams)				
	Body weight before *sleep deprivation*	Body weight after *sleep deprivation*	Weight loss after *sleep deprivation*	Body weight after *sleep recovery*	Weight gain after *sleep recovery*
I	241,67±30,28	-	-	-	-
II	241,67±25,82	216,67±20,41	25,00±15,81	-	-
III	245,83±18,82	229,17±36,80	16,67±20,41	-	-
IV	237,50±13,69	220,83±10,21	16,67±12,91	258,33±20,41	37,50±13,69
V	225,00±15,81	220,83±18,82	4,17±10,21	250,00±15,81	29,17±10,21

Description: Group I = healthy control group, Group II = PSD for 96 hours, Group III = TSD for 96 hours, Group IV = PSD for 96 hours continued with *sleep recovery*, Group V = TSD for 96 hours continued with *sleep recovery*.

Table 2. Average Parameter Of The Reproduction Quality Of Experimental Animals

Groups	Leydig cells number	Spermatogenic scores	Sertoli cells num
I	5,69 ± 0.70	8,89 ± 0,17*	40,02 ± 2,04
II	4,45 ± 0,57	7,90 ± 0,51	34,73 ± 1,63*
III	3,32 ± 0,54*	7,72 ± 0,37#	29,78 ± 1,87*
IV	5,91 ± 1,43	8,35 ± 0,85	38,38 ± 1,43
V	5,51 ± 0,58	8,27 ± 0,27	38,10 ± 1,63

* significantly different (p < 0,05) in *Post-HocTukey* test against other groups

significantly different (p < 0,05) in *Post-HocTukey* test against group IV and V

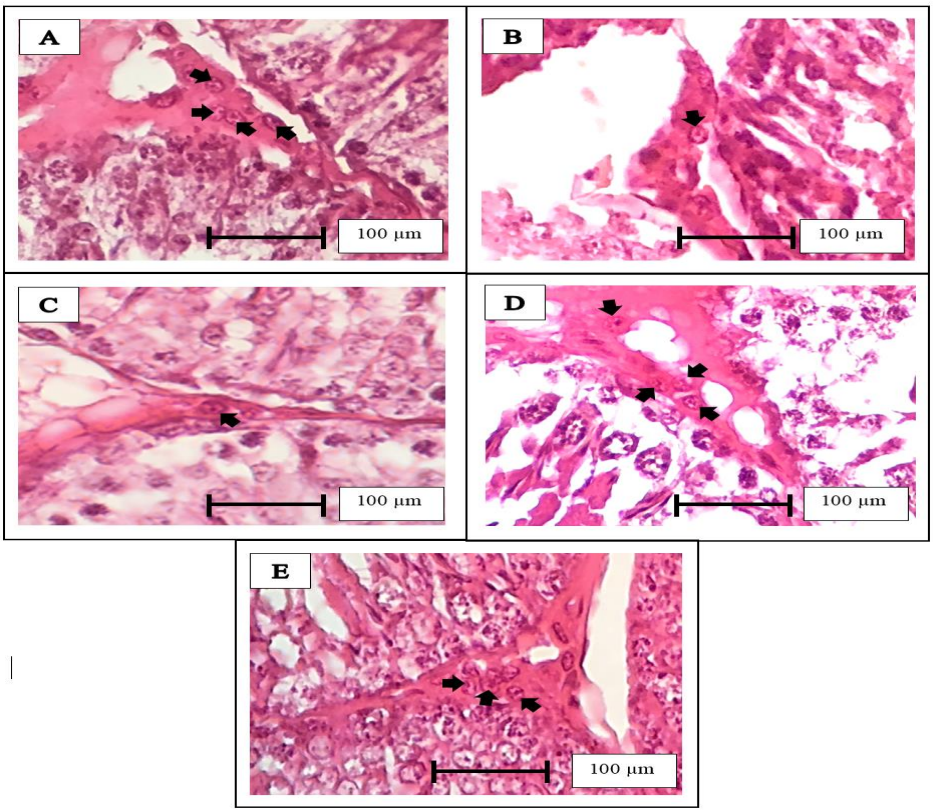

Figure 1. The Leydig Cells In The Interstitial Tissue Of Right Testis In Rattus Norvegicus. Leydig cells are round (polygonal), spherical core numbered one or two, large, and acidophi cytoplasm (black arrow). A = group I, group II B, C = group III, D = group IV, and E = group V (HE staining, 400X magnification, scale of 100 µm).

DISCUSSION

Group I as the control group had an unchaged mean weight groups at 241.67 ± 30.28 grams at the beginning and end of the study because in group I there was no exposure to the stress from outside resulting in weight loss and excessive energy expenditure in white rats. Average weight groups II and III are both decreased during the 96 hours of sleep deprivation stress treatment. Group IV and V experineced the decreasing of the average weight for the sleep deprivation treatment, then gaining weight again after recovery sleep.

The calculation result of the mean number of Leydig cells showed that a decline in the average number of Leydig cells after induction of sleep deprivation stress. Rats with stress exposed showed increased corticosterone corticosteron that have an impact on the occurrence of oxidative stress that triggers the inhibition of mRNA Leydig cells and Leydig cell differentiation process so that it resulted a decline in the number of Leydig cells.[9] Sleep deprivation increases the secretion of proinflammatory cytokines TNF-α and stress hormone corticosterone and norepineprin as well.[10] Other hormones that influence the decrease in the number of Leydig cell stress induced by the hormone FSH and LH. The decreasing of hormones FSH and LH affect the HPA axis with increased stress causes the adrenal cortex increases the secretion of adrenocorticotropin hormone (ACTH) and corticosterone thereby affecting the secretion of FSH and LH and consequently impaired development of Leydig cells.[11] Synergism between FSH and testosterone produced by the Leydig cells are required in maintaining the formation and maturation stages of spermatocytes and spermatids.[12]

Induction models of PSD stress caused mice undergoing physical and psychological stress due to loss of sleep (sleep deprivation)[11]. Stress due to reduced sleep time response of the body with increased release of glucocorticoid hormones. An increase in levels of the hormone corticosterone in rats after administration of stress induction for 72 hours as compared to normal controls.[13] Levels of corticosterone hormone increases between 20-60 ng / mL compared with normal controls. Induction of PSD also cause a decrease in the secretion of the hormone melatonin, which is an antioxidant substance, thereby increasing the oxidative stress and the accumulation of reactive oxsidative stress (ROS).[14] Melatonin inhibits the formation of hydroxyl radicals and the oxidation of nitrite which induced damage to the tissue.[15] The stress exposure causes highly autoinhibition of ROS to nicotinamide adeninedinucleotide phosphate (NADPH) production that is important in the regeneration of glutathione (GSH).[16] Testicular tissue membrane layer is rich in polyunsaturated fatty acids which can be damaged when the accumulation of ROS, it can be interpreted that the testicular tissue is the vulnerable tissue that encountered oxidative stress.[17]

Total sleep deprivation increases the activity of the sympathetic nerve, activating the hypothalamus pituitary adrenal axis, and improve biomarkers of inflammation. This activation is a key mediator of the acute stress, which have an impact on reproductive health, especially the health of Leydig cells[18]. Total sleep deprivation is one of the harmful stress that can negatively impact to the sexual organs, especially the testes[19]. The significant decline in testosterone occurs after induction of TSD for 96 hours. [18-20] A decrease in testosterone caused by the increased glucocorticoid hormones resulting from the stress so that the development and differentiation of Leydig cells inhibited and then induce apoptosis of Leydig cells.[21]

Sleep recovery can restore the production and activity of antioxidants in the testes and Leydig cell damage one of which is a ipeptide glutathione (GSH).[6] The main function of tripeptide glutathione is to reduce the formation of hydrogen peroxide and organic peroxide that occurs as a result of ROS.[22] Sleep recovery can improve the recovery of testicular tissue due to an increase in circulating hormones LH and testosterone.[16] and a decrease in the concentration of corticosterone hormone.[23] These events can be concluded that sleep recovery can restore the function of the HPA axis becomes normal again.[24]

On the number of Sertoli cells, the treatment of PSD stress for 96 hours and TSD for 96 hours can reduce the mean number of Sertoli cells significantly. In the group which was given sleep recovery showed there is no difference in the mean number of Sertoli cells greatly with the normal group which showed recovery sleep can restore the mean number of Sertoli cells approaching normal after a stressful sleep deprivation treatment. Activation of the HPA axis resulting in the hypothalamus produces CRH and AVP that stimulates the secretion of ACTH binding to the melanocortin type 2 receptor (MC2-R) on fasiculata zone adrenal cortex, activation of MC2-R stimulates the secretion glucocorticoid that affect to increase inhibitory effect on the secretion of GnRH by GnIH and decrease GnRH secretion causes suppression of secretion of FSH, LH and testosterone which play a role in cell proliferation Sertoli.[25-27]

The treatment group who received sleep recovery (groups IV and V) had a mean number of Sertoli cells higher than the PSD group (group II) and TSD without sleep recovery (group III) due to sleep recovery which resulting in decreased lipid peroxidation and free radical production. Glutathione is an antioxidant in the body showed increasing after sleep recovery thus inhibiting oxidative stress that plays a role in decreasing the number of cells Sertoli[6]. Group IV and V had a mean number of Sertoli cells that are not much different due to the same duration of the treatment of sleep recovery given that was for 96 hours, indicating that treatment of sleep recovery for 96 hours after the PSD nor the TSD for 96 hours gives the same effect on the number of Sertoli cells recovery in rats.

In the mean of spermatogenic score, healthy control group (I) showed the highest number compared to the treatment group. The decrease of sperm in TSD and PSD

stress models groups compared to the control group in the rat due to high levels of glucocorticoids which associated with low melatonin levels due to reduced sleep time.[8] The release of melatonin hormone by the pineal gland occurs optimally at night, especially at bedtime. Low levels of melatonin are responsible for the activation of the much glucocorticoid receptor in tissue.[25]

The high concentration of corticosterone in the hypothalamus responds to lower expression of gonadotropin releasing hormone (GnRH) and result in increased expenditures inhibiting gonadotropin hormone (GnIH) that inhibits secretion of LH, FSH and testosterone. Depletion of testosterone and decreased activity of gonadosomatic seminiferous tubules caused by low levels of LH and FSH, thus causing a disruption of spermatogenesis.[26] On the stress induction in animals suggest that there was a decrease of FSH by 1-2 ng / dL and LH of 0.1 to 0.3 ng/dL compared with controls[8]. There was a decrease of testosterone levels ±0.7 ng/dL post-induction of stress in 5 days, dropped to ±1 ng/dL post-induction of stress in 10 days and dropped to ±1.6 ng/dL after 15 days of stress induction[11]. The longer of sleep disorders, the greater the stress that occurs. Consequently it effects to a decrease in testosterone levels. This causes the TSD induction stress model group having decreased spermatogenic score more significant than the PSD stress model group[11]. Administration of stress for 72 hours increases corticosterone approximately 40-50 ng / dL and significant than given SD stress for 24 hours in rats[27] so that it showed the longer of sleep disorder, the greater the stress that occurs which influences to the decreased levels of testosterone hormone. This causes TSD induction stress model group had a decreased spermatogenic score more significant compared with PSD stress model group. [11]

Glucocorticoid binding with spermatogenic cells triggers the mitochondrial damage that decline the membrane potential. the loss of electron transport chain increases free electrons release.[28] These free electrons trigger the formation of free radicals and a decrease in main antioxidant in intratestikular, namely glutathione, superoxide dismutase, catalase and glutathione s- trasferase.[29-30] In male rats induced PSD for 72 hours showed an increase of lipid peroxides and decreased antioxidant glutathione.[31] The increase in free radicals accompanied by a decrease in antioxidants intratestikular leading to oxidative stress then triggers damage to lipids, proteins, DNA nucleus and mitochondrial DNA which stop the process of mitosis and increased spermatogenic cell apoptosis.[32] After giving the recovery of natural sleep for 3 days, serum concentrations of LH in rats showed a significant improvement, followed by an increase in testosterone levels after recovery sleep for 5 days. Sleep has an important effect on some components of the endocrine system. This relationship demonstrates the importance of the central nervous system (CNS) and sleep in regulating endocrine function.[33] The release of melatonin hormone by the pineal gland occurs optimally at night especially at bedtime so that sleep acts as a very effective antioxidant to fight free radicals.[25]

The conclusion was there is a significant difference in the mean number of Leydig cells, spermatogenic scores, Sertoli cells in male albino rats (R*attus norvegicus*) post-induction in various sleep deprivation stress model and stress model which is become the most influential in the decreased of histological parameter of reproduction quality is TSD stress model.

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