The Effect of Epinephrine Administration on the Level of Gonadotropin Hormones of Japan Strain Female Mice (*Mus Musculus*)

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Keywords: Epinephrine, FSH, LH.

Abstract: Physical, chemical, and psychological stressors can affect the pulsatile frequency and amplitude of GnRH; this is important for FSH and LH secretion. Excessive increase in pulsation can reduce and stop FSH and LH secretion. This study aimed to prove the effect of epinephrine to FSH and LH levels. This study was an experimental laboratory with a post-test only control group design. This study used 24 female mice (*Mus musculus*) consisting of 6 groups, namely 1 control group and 5 treatment groups based on differences in the administration of epinephrine of 0.001 mg/ml, 0.002 mg/ml, 0.003 mg/ml, 0.004 mg/ml, and 0.005 mg/ml applied every day for 20 days starting at the beginning of the pro-estrus cycle. The results were analyzed using the One-Way ANOVA continued Multiple Comparisons Bonferroni test. The results showed that the dose difference in the administration of epinephrine gave a significant difference ($p < 0.05$) on FSH and LH levels starting at 0.001 mg/ml and 0.002 mg/ml, 0.003 mg/ml, 0.004 mg/ml, 0.005 mg/ml with control. It can be concluded that chemical stressors (epinephrine) can reduce FSH and LH levels. It is recommended that further research adds measurements on the levels of estrogen and progesterone and the levels of catecholamine.

1 INTRODUCTION

During their life, human beings can always experience stress. If stress continues, it will cause interference with various body systems, one of which is the reproductive system. The function of the gonadal axis can change under certain conditions such as physical, chemical, and psychological stressors. This stressor can cause an imbalance in the hypothalamus – pituitary - ovarian axis. The reproductive system imbalance can be in the form of ovulation disorders or suppression. Reproductive disorders that occur can be menstrual disorders, which include menarche delay, a short and inadequate luteal phase, and even secondary amenorrhea; these can cause reversible infertility (Vander Borght and Wyns, 2018).

One of the reproductive disorders in women is hormonal disorders. Hormonal disorders can cause disruption in the process of development and formation of egg cells (ovum) through the process of oogenesis. Oogenesis occurs in the ovary through certain stages and is controlled by hormones, especially gonadotropin Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). This gonadotropin hormone is produced by the anterior pituitary gland through Gonadotropin stimulation Releasing Hormone (GnRH) from the hypothalamus (Barret *et al.*, 2012; Klein, 2014)

Stressors, be it physical, chemical, and psychological, can activate the sympathetic nervous system and adrenal response (Tanner, Sport and Gore, 2012). Activation of the sympathetic nervous system by stressors can cause the release of local norepinephrine (NE) neurotransmitters at postganglionic sympathetic nerve endings, while the sympathetic nervous system will stimulate the adrenal medulla so epinephrine will be released into the circulation (Bonert and Melmed, 2017). Epinephrine has a unique characteristic to modulate norepinephrine (NE), where the norepinephrine released will be duplicated and strengthened by epinephrine to reach the same place through circulation (Barret *et al.*, 2012; Klein, 2014)

Asman, A., Sinthania, D. and Marni, L.
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DOI: 10.5220/0009124301190123
In *Proceedings of the 2nd Health Science International Conference (HSIC 2019)*, pages 119-123
ISBN: 978-989-758-462-6

Stress can increase the production of glands or the epinephrine hormone. Under normal circumstances, hormones can have a positive effect, such as making us more motivated to work or make us more focused. However, excessive hormone production due to prolonged stress will lead to fatigue and even depression. Physical illness will also present easily, as a result of faster blood pumping, which disrupts the metabolism and the oxidation process in the body. The epinephrine hormone is synthesized in the medulla adrenal gland by chromatin cells (Gardner and Shobac, 2011)

Continuous stress can affect the frequency and amplitude of pulses of Gonadotropin-Releasing Hormone (GnRH), which is important for the secretion of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). In addition, stressors can also activate the sympathetic nervous system (release of norepinephrine) and the adrenal response (release of epinephrine). Increased levels of epinephrine and norepinephrine can increase the GnRH pulse. Excessive increase in pulsation can reduce and stop FSH and LH secretion. This reduction in FSH and LH will disrupt the oogenesis process (Gardner and Shobac, 2011; Hall and Guyton, 2011).

Normally GnRH is secreted through episodic pulses, which is important for normal FSH and LH secretions (Barret *et al.*, 2012). Studies show that changes in FSH and LH secretion require the pulsatile release of GnRH with a frequency of amplitude within the critical limit. This has been proven by research on monkeys who were administered 1 microgram of GnRH/minute for each hour (1 pulsation/hour)—the treatment results in ± 2 micrograms/ml concentration of GnRH in human portal blood. Increasing the frequency of GnRH pulses to 2 and 5 pulses/hour will stop gonadotropin secretion. Gonadotropin secretion will also decrease if the dose of GnRH is increased (Arief, 2006).

Experiments on female mice, by giving sub-cutaneous epinephrine at 0.001 mg can affect the pulsatile frequency and amplitude of GnRH, thereby disrupting FSH and LH production, which results in a decrease in the number of tertiary and deGraaf follicles in the ovary (Gardner and Shobac, 2011). Experiments on male mice by giving sub-cutaneous epinephrine at 0.001 mg, 0.005 mg, and 0.01 mg causes disruption in the process of spermatogenesis due to disruption in the secretion of GnRH in stimulating the production of FSH and LH (Arief, 2006)

Another study confirms the administration of epinephrine at 0.002 mg continuously for one cycle of spermatogenesis in mice (*Mus musculus*) reduces the quantity and quality of spermatozoa (Abdullah, 2008). Another study uses epinephrine at 0.001 mg, 0.005 mg, and 0.01 mg; the results show that administration at 0.001 mg alone has significantly reduced the number of spermatogenic cells (Utami, 2010). This is because of the disruption of hormonal arrangements in the hypothalamic-pituitary-testicular axis pathway.

According to a study in fertility and sterility, scientists from the University of California have discovered the negative effects of stress on the possibility of pregnancy on women. The stress felt by participants in the study turned out to affect the number of eggs and embryos produced—too much stress means fewer eggs produced and fertilized. Women who are easily offended, angry, and depressed have fewer eggs (Aggarwal *et al.*, 2013).

Based on the aforementioned description, this study was conducted to determine the effect of epinephrine on gonadotropin levels of female mice (*Mus musculus*).

2 METHODS

The research was a laboratory experiment using a post-test only control group. Treatment of experimental animals was done at the Pharmacy Laboratory, Faculty of Pharmacy of Andalas University, Padang. Examination of FSH and LH hormone levels was conducted at the Laboratory of Biochemistry, Faculty of Medicine of Andalas University, Padang. The research was conducted for six months. The population of this study was female mice (*Mus musculus*) from the Pharmacy Laboratory, Faculty of Pharmacy of Andalas University, Padang. The study needed 24 mice, yet due to the possibility of death, we had five mice in each of the six groups, so we kept 30 mice in total.

3 RESULTS AND DISCUSSION

The level of FSH hormone on female mice (*mus musculus*) of the control group and treatment groups at the proestrus phase.

Table 1 shows that the average FSH hormone level in the control and treatment groups decreases—the more epinephrine administered, the smaller the average is. The normality test (Kolmogorov Smirnov) shows that data was normally distributed

so parametric tests (ANOVA) could be done. Based on the results of the statistical test, there was a significant difference between the control and treatment groups ($p < 0.05$). Therefore, the statistical test continued with the Multiple Comparisons (post hoc test) employing the Bonferroni test type and the results are presented in Table 2.

Table 2 shows that the average between the control group and the treatment group is different.

The higher the dose of epinephrine administered, the bigger the difference in the average is. The difference was significant starting with epinephrine dose of 0.001 mg ($p<0.05$). The administration of epinephrine of 0.002 mg, 0.003 mg, 0.004 mg, and 0.005 mg also gave a significant difference.

Table 1: The level of FSH hormone on female mice (*Mus musculus*) of the control group and treatment groups at the proestrus phase.

Treatment	Repetition				Average (mIU/ml)	SD
	1	2	3	4		
K [Control]	0.720	0.736	0.776	0.844	0.769	0.055
P1 [0.001]	0.332	0.342	0.360	0.378	0.353	0.020
P2 [0.002]	0.390	0.308	0.322	0.342	0.340	0.035
P3 [0.003]	0.300	0.322	0.360	0.360	0.335	0.029
P4 [0.004]	0.368	0.342	0.262	0.322	0.323	0.045
P5 [0.005]	0.280	0.332	0.368	0.308	0.322	0.037

Table 2: The Results of the multiple comparisons of FSH hormone levels between the control and treatment groups.

Control	Epinephrine Dose (mg)	Average Difference (mIU/ml)	p-value
Control	[0.001]	0.416	0.000
	[0.002]	0.428	0.000
	[0.003]	0. 433	0.000
	[0.004]	0.445	0.000
	[0.005]	0.447	0.000

Table 3: The level of LH hormone on female mice (Mus musculus) of the control group and treatment groups at the proestrus phase.

Treatment	Repetition				Average (mIU/ml)	SD
	1	2	3	4		
K [Control]	0.304	0.354	0.364	0.392	0.353	0.036
P1 [0.001]	0.198	0.158	0.182	0.218	0.189	0.025
P2 [0.002]	0.158	0.182	0.182	0.198	0.180	0.016
P3 [0.003]	0.170	0.190	0.170	0.182	0.178	0.009
P4 [0.004]	0.158	0.172	0.170	0.182	0.170	0.009
P5 [0.005]	0.135	0.182	0.140	0.182	0.159	0.025

Table 4: The results of the multiple comparisons of LH hormone levels between the control and treatment groups.

Control	Epinephrine Dose (mg)	Average Difference (mIU/ml)	p-value
Control	[0.001]	0.164	0.000
	[0.002]	0.173	0.000
	[0.003]	0. 175	0.000
	[0.004]	0.183	0.000
	[0.005]	0.193	0.000

The level of LH hormone on female mice (*Mus musculus*) of the control group and treatment groups at the proestrus phase.

Table 3 shows that the average LH hormone level in the control and treatment groups decreases—the more epinephrine administered, the smaller the average is.

The normality test (Kolmogorov Smirnov) shows that data was normally distributed so parametric tests (ANOVA) could be done. Based on the results of the statistical test, there was a significant difference between the control and treatment groups ($p < 0.05$). Therefore, the statistical test continued with the Multiple Comparisons *(post hoc test*) employing the Bonferroni test type and the results are presented in Table 4.

Table 4 shows that the average between the control group and the treatment group is different. The higher the dose of epinephrine administered, the bigger the difference in the average is. The difference was significant starting with epinephrine dose of 0.001 mg ($p<0.05$). The administration of epinephrine of 0.002 mg, 0.003 mg, 0.004 mg, and 0.005 mg also gave a significant difference.

The effect of epinephrine administration on FSH hormones levels.

The results of the study confirm that the FSH hormone levels of mice decreased. The higher the dose of epinephrine administered, the lower the FSH hormone level produced was. This shows that the administration of epinephrine with a dose of 0.001 mg has begun to influence the FSH hormone levels. The administration of epinephrine at 0.002 mg, 0.003 mg, 0.004 mg, and 0.005 mg also influenced the FSH hormone levels.

The effect of epinephrine administration on LH hormones levels.

The results of the study confirm that the LH hormone levels of mice decreased. The higher the dose of epinephrine administered, the lower the LH hormone level produced was. This shows that the administration of epinephrine with a dose of 0.001 mg has begun to influence the LH hormone levels. The administration of epinephrine at 0.002 mg, 0.003 mg, 0.004 mg, and 0.005 mg also influenced the LH hormone levels.

This means that the administration of stressors using low epinephrine levels has caused pulsatile changes in GnRH in the hypothalamus that exceeds the critical limit; this results resulting in a significant decrease in GnRH production leading to down-regulation of the anterior pituitary that finally causes a decrease in FSH and LH secretion (Guyton and Hall, 2014).

Repeated epinephrine administration as stressors during the study caused the activation of the sympathetic nervous system, which could cause the release of local norepinephrine neurotransmitters at the postganglionic sympathetic nerve end, while the sympathetic nervous system would stimulate the adrenal medulla so epinephrine would be released into the circulation. GnRH pulsation control can be influenced by catecholaminergic (epinephrine and norepinephrine). Catecholamine may be working by changing the frequency (and possibly amplitude) of GnRH release. Increasing the pulse frequency and amplitude of GnRH can reduce and stop gonadotropin secretion. Finally, it will suppress FSH and LH secretion (Hall and Guyton, 2011; Barret *et al.*, 2012).

Repeated and prolonged epinephrine administration will also cause activation of the amygdala in the limbic system. This system will stimulate the release of hormones from the hypothalamus, namely Corticotropic Releasing Hormone (CRH). This hormone will directly inhibit hypothalamic GnRH secretion. The result is reduced stimulation to the anterior pituitary leading to reduced FSH and LH secretion (Hall and Guyton, 2011).

Hormonal regulation of female reproductive function occurs in a pathway called the hypothalamus-pituitary-ovarian axis. This regulatory mechanism starts from the hypothalamus gland with the release of GnRH. Then, GnRH will stimulate the anterior pituitary gland and the stimulation will make the anterior pituitary to release the FSH and LH hormone. FSH and LH hormone will then stimulate the ovaries to produce estrogen and progesterone (Barret *et al.*, 2012).

4 CONCLUSIONS

Stressor using epinephrine administered repeatedly will affect the pulsatile frequency and amplitude of GnRH, which is important for FSH and LH secretion. Research suggests that repeated administration of subcutaneous epinephrine at 0.001 mg causes a decrease in the number of tertiary and deGraaf follicles in the ovary of mice caused by disruption of the hypothalamic-pituitary- ovary axis (Gusty, 2007). Other study also confirms that the administration of epinephrine at a dose of 0.001 mg

has reduced the number of primary follicles in the ovary of mice (Utami, 2010).

One study confirms that the administration of epinephrine at 0.002 mg continuously for 1 cycle of spermatogenesis in mice (*Mus musculus*) reduces the quantity and quality of spermatozoa (Abdullah, 2008). Another study uses epinephrine at 0.001 mg, 0.005 mg, and 0.01 mg; the results show that administration at 0.001 mg alone has significantly reduced the number of spermatogenic cells (Trussell, Kunselman and Legro, 2010). This is because of the disruption of hormonal arrangements in the hypothalamic-pituitary- testicular axis pathway.

The results of this study are also in accordance with the research conducted by (Arief, 2006), where the administration of epinephrine at 0.001 mg and 0.005 mg and 0.01 mg causes a significant effect on the process of spermatogenesis. The stressors given affect the frequency and amplitude of the hypothalamus so the regulation of hormonal secretions in the hypothalamic-pituitary-testicular axis does not occur harmoniously.

ACKNOWLEDGEMENTS

The authors thanks Aulia Asman, Debby Sinthania, and Linda Marni for their dedicated work in collecting data used in this article as a part of the objective of our research for the effect of epinephrine administaration on the level of gonadotropin hormones of Japan strain female mice *(Mus Musculus)*.

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