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THE EFFECT OF L-ARGININE TO THE NUMBER OF SERTOLI CELLS AND LEYDIG CELLS OF MICE (*MUS MUSCULUS*) AFTER EXPOSURETO HIGH TEMPERATURE

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ABSTRACT

The purpose of this study was to find out the effect of L-arginine on the number of Sertoli cells and Leydig cells in mice (*Mus muculus*) after exposure to high temperature. The subjects of this study were 30 male mices, 8 weeks old. This research was conducted by using Randomized Block Design experimental group 2x3 factorial design with 5 replications. The negative control group was given aquabidest without heat stress, while treatments group 1 and 2 were given L-arginine at a dose 1.3 mg/kg BW and 2.6 mg/kg BW. The positive control group was given aquabidest with high temperature exposure at 40° C, while treatment groups 3 and 4 were given high temperature exposure at 40 °C with L-arginine at a dose 1.3 mg/kg BW and 2.6 mg/kg BW. Sertoli cells and Leydig cells were calculated and analyzed using two way ANOVA followed by Duncan Test. The results of this study were revealed that the treatment of heat temperature can reduce the number of Sertoli cells and Leydig cells in mice (pˆ0.05). The treatment with L-arginine orally to mice can increase the number of Sertoli cells with a dose 1.3 mg and Leydig cells with a dose 2.6 mg (pˆ0.05). This study concluded that there is no interaction between the heating temperature and the doses of L-arginine to the number of Sertoli cells and Leydig cells in mice (pˆ0.05).

KEY WORDS : L-arginine, Sertoli Cells, Leydig Cells

INTRODUCTION

Spermatogenesis involves Sertoli cells which function to provide nutrition to spermatogenic cells, as well as Leydig cells in interstitial tissue that function to produce testosterone hormone for maturation of spermatozoa cells (Heryani *et al*., 2011). The process of spermatogenesis is influenced by several factors such as hormonal factors, inhibiting factors of epididymal function, radiation factors, and temperature factors. Spermatogenesis at normal testicular temperatures is 35 °C. Spermatogenesis will be inhibited if there is an increase of several degrees from the normal temperature of the testes (Ermiza, 2013). Heat exposure causes damage to the Sertoli cell membrane and Leydig cells (Arimbi *et al.,* 2015). Damage to Sertoli cells and Leydig cells will affect the work of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and testosterone (Murray *et al.,* 2009); causing disruption of the process of spermatogenesis in the seminiferous testicular tubules. In previous studies, environmental conditions with a temperature of 40 °C for 60 minutes can cause abnormalities in cells in the testicular organs (Ermiza, 2013).

One effort to increase livestock productivity can be done by developing a maintenance business (Tjatur and Ihsan, 2011), by providing good food and sanitation. L-arginine is a non-essential amino acid that plays a role in the body's defense system and cellular immunity.L-arginine also plays an active role in the process of spermatozoa formation (Srivastava *et al.,* 2006). L-arginine can increase metabolic activity and increase the availability of energy in cells so that the process of spermatogenesis and spermatozoa quality becomes better (Aziz, 2015). In previous studies, L-arginine

could have an effect on Sertoli cells and Leydig cells in mice. L-arginine functions as a form of GnRH produced by hypothalamus, causing release of FSH and LH. FSH functions to mature spermatozoa cells by Sertoli cells and regulates spermatogenesisin the testicular seminiferous tubules. FSH plays a role in the formation of Sertoli cells and produces the elements needed in the process of spermatogenesis. LH functions in stimulating Leydig cells for the synthesis of the hormone testosterone to control the development of the reproductive organs needed in spermatogenesis for cells division and control the development of male secondary sex. Simultaneously, the hormone testosterone diffuses into testicular seminiferous tubules has a strong effect on the process of spermatogenesis (Mahidin *et al.*, 2018). The content of Nitric Oxide (NO) from the synthesis of L-arginine also plays a role in the reproductive system (Utama, 2017). Nitric Oxide in the testes can affect testosterone synthesis, seminiferous tubule contraction and germinal metabolism. Nitric Oxide affects Leydig cells in the subclasses form called Testis Neuronal Nitric Oxide Synthase (TnNOS) then will involved in the process of steroidogenesis (Aziz, 2015).

Therefore, it is necessary to give L-arginine as a safe sexual nutrition to improve reproduction health (Appleton, 2002). The ability of spermatozoa to fertilize an egg mainly depends on spermatozoa motility and membrane integrity. L-arginine plays an important role in regulating the body's defenses, and also plays an active role in the process of spermatogenesis. L-arginine deficiency can cause metabolic disorders in spermatozoa, causing a decrease in spermatozoa motility and interfere the process of spermatogenesis (Srivastava *et al.*, 2006).

MATERIALS AND METHODS

This research was conducted at the Experimental Animal Laboratory Unit, Faculty of Veterinary Medicine, Universitas Airlangga. Histopathological preparation was conducted at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

The research materialsare 30 male mice (*Mus musculus*) 20-30 g body weight and L-arginine with SIGMA ALDRICH®contain of Arginine 98%. The tools used are four light bulbs with 15 watts of power for heat exposure on mice, mouse cage boxes, thermometers, minor surgical instruments, glass objects along with glass covers and microscope.

Mice were divided into 6 groups, groups without heat stress and groups with heat stress. Making heat stress using a cardboard box with two 15-watt light bulbs, two thermometers to measure the temperature of heat that will be given to mice with a temperature of 40 °C, and carried out monitoring for one hour every 5 minutes to control the degree of heat.

The administration of a 500 mg dose of L-arginine to humans was converted to a dose of animal mice with a conversion factor of 0.0026 (Pitaloka, 2016). The treatment was carried out for 35 days because spermatogenesis in male mice required 35.5 days.

The dosage of L-arginine used each time per treatment is as follows:

Doses of treatment P_0A_1 and P_1A_1: 500 mg x 0.0026 = 1.3 mg

Doses of treatment P_0A_2 and P_1A_2: 500 mg x 0.0026 x 2 = 2.6 mg

The following are the stages of treatment for each treatment:

P_0A_0: negative control treatment group where mice do notplaced in a high temperature room, not given L-arginine, only given aquabidest.

P_0A_1: mice treatment group which is conditioned not in the high temperature room and given L-arginine at a dose of 1.3 mg/day.

P_0A_2: mice treatment group which is conditioned not in the high temperature room and given L-arginine at a dose of 2.6 mg/day.

P_1A_0: positive control treatment group in which the mice are conditionedin a high temperature room and not given L-arginine, but only given aquabidest.

P_1A_1: mice treatment group which is conditioned in the high temperature room and given L-arginine at a dose of 1.3 mg/day.

P_1A_2: treatment group of mice that are conditioned in a hight temperature room and given L-arginine at a dose of 2.6 mg/day.

Research data on the effect of L-arginine administration on the number of Sertoli cells and Leydig cells of mice (*Mus musculus*) after exposure to high temperatures were collected and the average was taken for data analysis using Two Way ANOVA.

RESULTS AND DISCUSSION

This research was conducted to determine the effect of L-arginine administration on the number of Sertoli cells and Leydig cells of mice (*Mus musculus*) after exposure to high temperatures.

In the group treated with high temperatures, Sertoli cells experienced a lot of lysis caused by heat temperatures that cause damage to cells. Oxidative stress on cell membranes will cause damage to the Sertoli cell membrane. Damage to the membrane can deactivate the membrane binding with receptors or enzymes that can disrupt the normal function of cells. Damage to Sertoli cells will affect the work of FSH. If FSH decreases, there is a decrease in activity of spermatogenesis so that it affects fertility (Mahidin *et al.*, 2018).

Table 1. Total number of Sertoli cells of Mice (*Mus musculus*) after exposure of high temperature

Treatment	Mean ± SD	
P_1	High temperature	6.69^a ±1.1
P_0	No high temperature	7.48^b ±1.1

Note: Different superscripts in each column show significant differences (p<0.05).

The effect of L-arginine which produces Nitric Oxide (NO) through Nitric Oxide Synthase (NOS) can affect lamina propia and influence the transport of nutrients into the lumen of the tubules (Middendorff *et al.*, 1997). The number of Sertoli cells that treated with L-arginine in different doses is shown in the Table 2 below.

Table 2. The number of Sertoli cells of mice (*Mus musculus*) in different treatment doses of L-arginine

Treatment		Mean ± SD
A0	Dose L-arginine 0 mg	5.56a ± 1.35
A1	Dose L-arginine 1,3 mg	7.50b ± 1.35
A2	Dose L-arginine 2,6 mg	8.20b ± 1.35

Note: Different superscripts in each in the same column show significant differences (p<0.05).

Nitric Oxide synthesis is needed for maintenance of FSH secretion. This is due to the fact that Nitric Oxide can activate guanylate cyclase thereby increasing thec-GMP synthesis which is responsible forreleasing FSH (Pitaloka, 2016). Arginine also functions as a Gonadothropin Releasing Hormone (GnRH) form that produced by the hypothalamus which caused the release of FSH and LH. FSH functions as sperm maturation by Sertoli cells in the testes. FSH influences Sertoli cells to grow and produce elements needed in spermatogenic processes (Mahidin *et al.*, 2018).

The effect of heat stress on the number of Leydig cells showed a significant difference in the Table 3 above. This occurs due to metabolic disorders in Leydig cells that cause lipid peroxidation, DNA damage due to oxidative stress. and failure of ATP formation. The failure of the formation of ATP causes anaerobic glycolysis and mitochondria, and causes the accumulation of lactic acid so that it affects the decrease in intracellular pH. A decrease in intracellular pH causes nuclear clumping, and it will continue to interfere with RNA synthesis and result in cell necrosis (Arimbi *et al.*, 2015).

Table 3. Total number of Leydig cells of Mice (*Mus musculus*) after exposure of high temperature

Treatment	Mean ± SD	
P0	No high temperature	14.41a ±3.5
P1	High temperature	11.41b ±3.5

Note : Different superscripts in each in the same column show significant differences (p<0.05).

Giving L-arginine to the number of Leydig cells in mice showed a significant difference that mentioned in the Table 4 below.

Table 4. The number of Leydig cells of mice (*Mus musculus*) in different treatment doses of L-arginine

Treatment		Mean ± SD
A0	Dose L-arginine 0 mg	11.00a ± 4.3
A1	Dose L-arginine 1,3mg	13.14ab ± 4.3
A2	Dose L-arginine 2,6 mg	14.60b ± 4.3

Note: Different superscripts in each in the same column show significant differences (p<0.05).

Arginine can increase the testosterone hormone which functions to control the development of the reproductive organs in the process of spermatogenesis, which is required in cells division for spermatozoa formation and control secondary sex development in males. Arginine also functions as a Gonadothropin Releasing Hormone (GnRH) formation produced by the hypothalamus which will cause the LH release. LH functions to stimulate Leydig cells for testosterone synthesis. Simultaneously, the hormone testosterone produced will diffuse into the seminiferous tubules (Mahidin., 2018)

CONCLUSION

After researching the effect of L-arginine on the number of Sertoli cells and Leydig cells of mice (*Mus*

musculus) after exposure of high temperature, it can be concluded that heat stress can reduce the number of Sertoli cells and Leydig cells of mice. Whereas L-arginine can increase the number of Sertoli cells and Leydig cells on mice. From this study, it concluded that there was no interaction between L-arginine and heat stress on the number of Sertoli cells and Leydig cells in mice.

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