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The Effect of Epinephrine On The Development of oogenesis Of Mice (*Mus Musculus*) Strain of Japanese

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Original Article

ABSTRACT

When stress persists continuously and repeatedly, it will automatically increase the epinephrine in the body in which excessive consequently can provide interference on various body systems. In the event of physical stressors can affect the frequency and amplitude of pulsatile gonadotropin-releasing hormone (GnRH). It is important for the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Additionally stressors can also activate the sympathetic nervous system. If the increase is excessive pulsation can reduce and stop the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Decrease in Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) will inhibit the growth of ovarian follicles and decrease the synthesis of estrogen and progesterone in the ovaries. Decreased synthesis of estrogen and progesterone can cause a decrease in the number of ovarian follicles (Speroff, 1994).

The study population was female mice derived from laboratory Biomedic Andalas University in Padang. Mice used were 2-3 months old, weighing an average of 25-35 grams.

The Effect of Epinephrine on the Development of Oogenesis of Mice (*mus musculus*) Strain of Japanese, is the growth of primary follicles in which a decline in the number of primary follicles ranging from provision of 0.002 mg / ml, epinephrine administration lowered formation of secondary follicles at a concentration of 0.004 mg / ml and above but no decrease in concentration of 0.002 mg / ml, epinephrine administration lowered formation of tertiary follicles at a concentration of 0.004 mg / ml, 0.006 mg / ml, 0.008 mg / ml and 0.01 mg / ml and no decrease in concentration of 0.002 mg / ml, epinephrine administration did not reduce the formation of follicle de Graaf and administration of epinephrine significantly reduce the formation of the corpus luteum at a concentration of 0.004 mg / ml, 0.006 mg / ml, 0.008 mg / ml and 0.01 mg / ml and no decrease in concentration of 0.002 mg / ml.

Keywords: epinephrine, oogenesis, mice

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Introduction

Repeated stressors can cause stress. Epinephrine injection is a severe form of representation given stressor repeatedly. Expenditure due to stress is the occurrence of excessive epinephrine and Norepinephrine, epinephrine levels so high in the blood Norepinephrine (Cunningham, 2002). Elevated levels of epinephrine will increase the stimulation of the hypothalamus, an increase in corticotropin-releasing hormone (CRH) (Rajakoski, 1960). In the situation of 1) an increase in excessive stimulation or prolonged stress on the hypothalamus, adrenal cortex is not able to respond to the increased secretion of Adrenocorticotrophic hormone (ACTH) and 2) a cortisol increased spending, and of even divert some cholesterol precursors into the androgen pathway, the

result is: an increase Dehydroepiandrosterone (DHEA), this does not inhibit androgen excess Adrenocorticotrophic hormone (ACTH) but inhibits gonadotropin, which will reduce the secretion of follicle-stimulating hormone (FSH) and LH (Sherwood, 2001).

Normally GnRH is secreted in episodic pulsations. In the present study can be shown that changes in FSH and LH secretion is pulsatile GnRH requires expenditures by the frequency amplitude in the critical limit (Speroff, 1994). However, when the pulsation amplitude and frequency of GnRH increased excessively can reduce and stop the secretion of gonadotropins. Excessive increase in GnRH pulse frequency will increase GnRH pulse into 2 and 5 pulsation / hours and will stop the secretion of gonadotropins. Secretion of gonadotropins

also will drop when the dose of GnRH is increased. Increased GnRH pulse will stimulate an increase in concentrations of LH and FSH. This increase will lead to the setting down (down regulation) that can trigger the internalization process, which means the loss of membrane receptors and the reduction of biological functions (Gougeon, 1993).

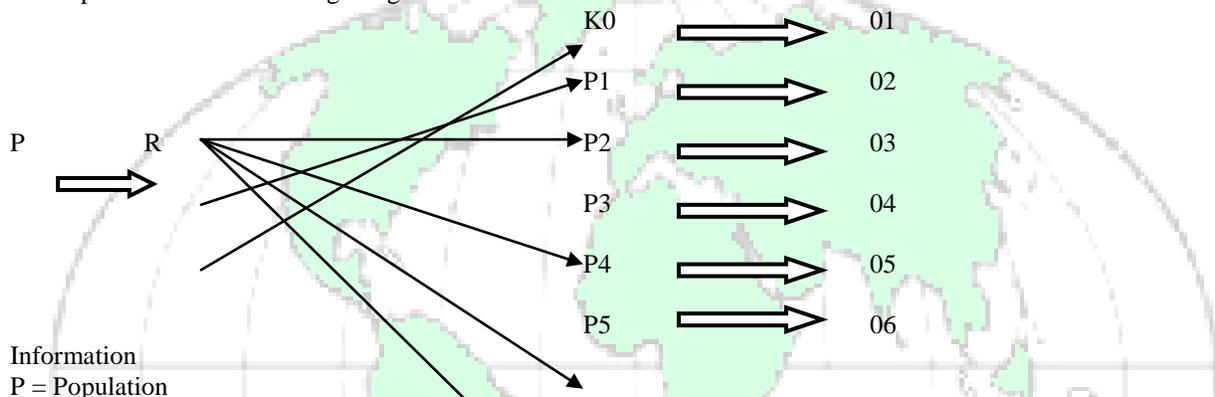
Oogenesis depends on the co follicle stimulating hormone (FSH) and LH from the pituitary on ovarian cells. LH affects the interstitial cells are produced in cells of the anterior pituitary kromofob, function triggers the development of follicles (theca interna cells and granulose cells) and also trigger ovulation at mid-cycle (LH-surge), which is required for normal development of the line lineage-oogenesis. Decrease in

FSH and LH would affect cells kromofob hold follicle development and ovulation trigger, so that the function of the corpus luteum produces progesterone bit-cell and theca interna cells produce less estrogen, thus oogenesis will be disrupted and result in lower quality and quantity of follicles produced (Sherwood, 2001).

Methodology

The study design: This type of research is done is by using laboratorial experimental study designs post test only control group design.

Draft Research: In the schematic design of the study can be described as follows:



Information

P = Population

R = randomization

K0 = control group not given the aqua bidest

P1 = Group 1 by administering injections of epinephrine subcutan [0.002 mg / ml] of 0.26 ml/20 g BW

P2 = Group 2 by administering injections of epinephrine subcutan [0.004 mg / ml] of 0.26 ml/20 g BW

P3 = Group 3 by administering injections of epinephrine subcutan [0.006 mg / ml] of 0.26 ml/20 g BW

P4 = Group 4 by administering injections of epinephrine subcutan [0.008 mg / ml] of 0.26 ml/20 g BW

P5 = Group 5 by administering injections of epinephrine subcutan [0.01 mg / ml] of 0.26 ml/20 g BW

01 = Data postest control group

02 = Data postest group 1

03 = Data postest group 2

04 = Data postest group 3

05 = Data postest group 4

06 = Data postest group 5

Explanation of dose: (ETRI, 2008) for each group.

I. $0.2 \text{ mg} \times 0.0026 = 0.00052 \times 50 = 0.26$ 0.1For concentrations of 0, 002 mg / ml

II. $0.4 \text{ mg} \times 0.0026 = 0.00104 \times 25 = 0.26$ 0.1For concentrations of 0, 004 mg / ml

III. $0.6 \text{ mg} \times 0.0026 = 0.00156 \times 16.75 = 0.26$ 0.1For concentrations of 0, 006 mg / ml

IV. $0.8 \text{ mg} \times 0.0026 = 0.00208 \times 12.5 = 0.26$ 0.1For concentrations of 0, 008 mg / ml

V. $1 \text{ mg} \times 0.0026 = 0.0026 \times 10 = 0.26$ 0.1For concentrations of 0, 01 mg / ml

Results

From the results of research on the effects of epinephrine on the development of oogenesis of mice

(Mus musculus) Strain Japan, the data obtained from various parameters i.e the development of primary follicle, secondary follicle, tertiary follicle, the

follicle de Graaf and KL, are shown in the tables below.

Prior to the analysis of the effect of epinephrine on the development of oogenesis do the normality test is non-parametric test of Kolmogorov-Smirnov and Shapiro Wilk. (The structure of this phrase is incorrect. We do not understand the subject and the

verb and the complement).

It turns out that for the test for normality with the Kolmogorov-Smirnov and Shapiro Wilk, we have not abnormal data. Then normality test followed by transformed by \sqrt{n} , can be seen in the table below (Table 2)

Table 1. Normality Test To Follicle Cells In Mice oogenesis Female, (Mus musculus) strain of Japanese

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
Primer F	,132	24	,200(*)	,918	24	,054
Seknder F	,195	24	,019	,887	24	,012
Trtier F	,130	24	,200(*)	,934	24	,122
Graff F	,519	24	,000	,393	24	,000
Lteum F	,265	24	,000	,795	24	,000

Table 2 Test Normality After Do With \sqrt{n} In Follicle Cells Female Mice (Mus musculus) strain of Japanese

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
$\sqrt{n1}$,180	24	,042	,890	24	,013
$\sqrt{n2}$,185	24	,032	,883	24	,010
$\sqrt{n3}$,169	24	,076	,890	24	,013
$\sqrt{n4}$,519	24	,000	,393	24	,000
$\sqrt{n5}$,301	24	,000	,792	24	,000

After standardization of the data obtained with rooted and re-tested for normality, obtained results are also not normally distributed. To this then tested the effect of epinephrine on the development of oogenesis of mice (Mus musculus) strain of Japanese with non-

parametric test of Kolmogorov-Smirnov and Kruskal-Wallis Test. (The structure of this phrase is incorrect. We do not understand the subject and the verb and the complement).

Table 3. Effect of Various Dose Epinephrine to Total Primary follicles in female mice (Mus musculus) strain of Japanese

Kontrol	Dosis Epinefrin (mg/ml)				
	0,002	0,004	0,006	0,008	0,01
Kolmogorov-Smirnov	1,414	1,414	1,414	1,414	1,414
P (P < 0,05)	0,037	0,037	0,037	0,037	0,037

Table 3. was based on the number of primary follicles as a result of epinephrine significantly different ($p < 0.05$) at various doses of control.

Table 4. Effect of Various Dose Epinephrine to Total Secondary follicles in female mice (Mus musculus) strain of Japanese

Kontrol	Dosis Epinefrin (mg/ml)				
	0,002	0,004	0,006	0,008	0,01
Kolmogorov-Smirnov	1,061	1,414	1,414	1,414	1,414
P	0,211	0,037	0,037	0,037	0,037

P < 0,05

Apparently the number of secondary follicles was significantly different due to epinephrine administration in various doses of control (except for the concentration of 0.002 mg / ml).

Table 5 Effect of Various Dose Epinephrine to Total Tertiary follicles in female mice (Mus musculus) strain of Japanese

Kontrol	Dosis Epinefrin (mg/ml)				
	0,002	0,004	0,006	0,008	0,01
Kolmogorov-Smirnov	1,061	1,414	1,414	1,414	1,414
P	0,211	0,037	0,037	0,037	0,037

P < 0,05

Apparently the number of tertiary follicles as a result of epinephrine were significantly different at various doses of control (except for the concentration of 0.002 mg / ml).

Table 6. Effect of Various Dose Epinephrine to Total De Graaf follicles in female mice (Mus musculus) strain of Japanese

Kontrol	Dosis Epinefrin (mg/ml)				
	0,002	0,004	0,006	0,008	0,01
Kolmogorov-Smirnov	0,354	0,707	0,707	0,707	0,707
P	1,000	0,699	0,699	0,699	0,699

Based on Table 6 of various doses of epinephrine has no effect on follicle de Graaf concentrations ranging from 0.002 mg / ml

Table 7. Effect of Various Dose of Epinephrine to Total corpus luteum in female mice (Mus musculus) Strain Japan (write again this phrase. We can't understand the signification)

Kontrol	Dosis Epinefrin (mg/ml)				
	0,002	0,004	0,006	0,008	0,01
Kolmogorov-Smirnov	1,061	1,414	1,414	1,414	1,414
P	0,211	0,037	0,037	0,037	0,037

P < 0,05

Apparently the amount of the corpus luteum of epinephrine were significantly different due to various doses of control (except for the concentration of 0.002 mg / ml).

Table 8. Provision of Various Dose Epinephrine Against Oogenesis in female mice(Mus musculus) strain of Japanese

	F.Primer	F.Sekunder	F.Tertier	F.De Graaf	C.Luteum
P	0,000	0,001	0,001	0,176	0,005

From Table 8, the provision of various doses of epinephrine effect on oogenesis, excepted in the follicle de Graaf, from the research results can be seen the influence of epinephrine on primary follicle, secondary follicle, tertiary follicle, the follicle and corpus luteum de Graaf, like the image below:

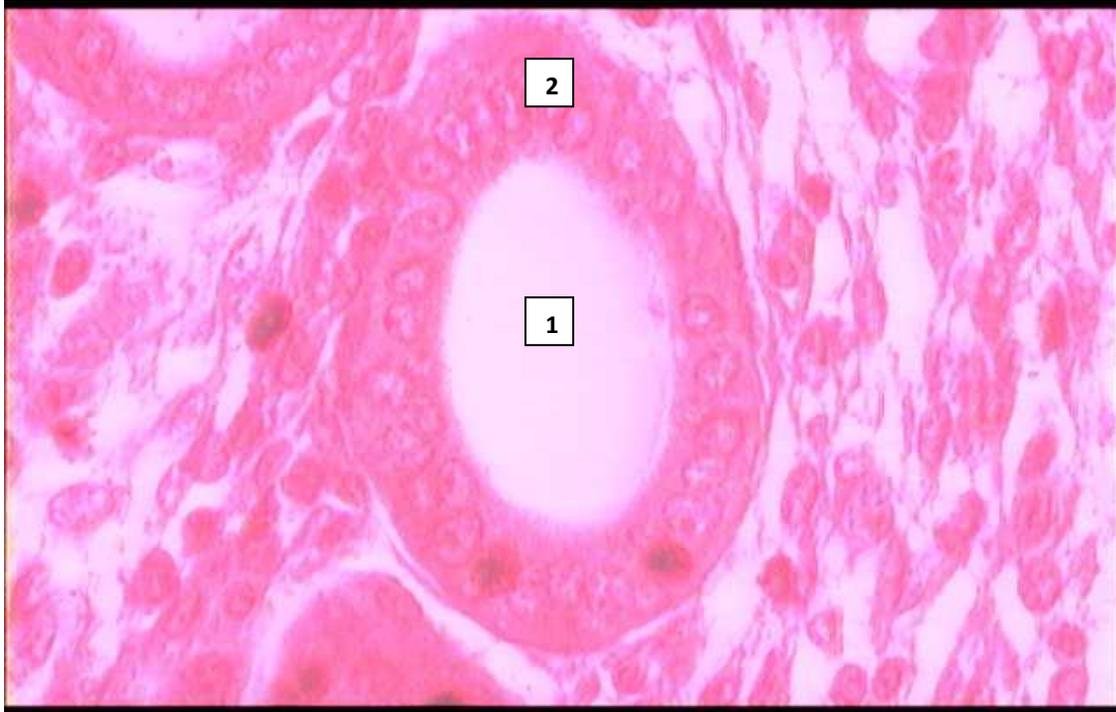


Figure1. Primary follicles (1. Oocytes and 2. Granulosa cells) are found the ovary, (HE, 400 X).

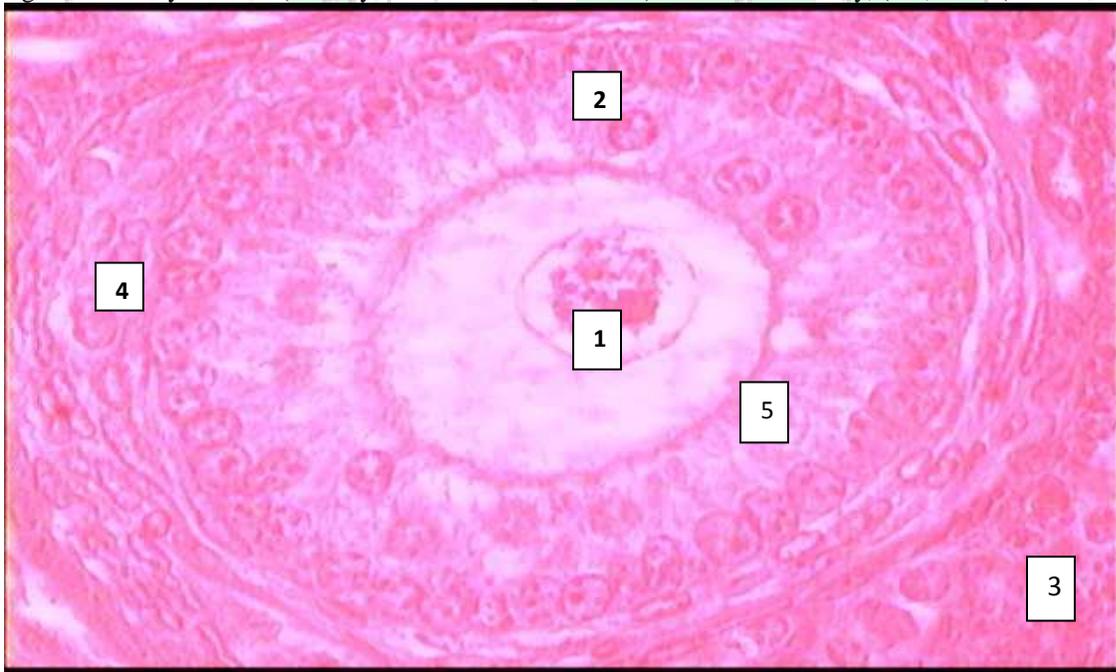


Figure2. Secondary follicles (1.oocyte, 2.granulosa cells, 3.theca externa, 4.theca internal, 5.zona pellucida) which is found in the ovaries, (HE, 400 X)

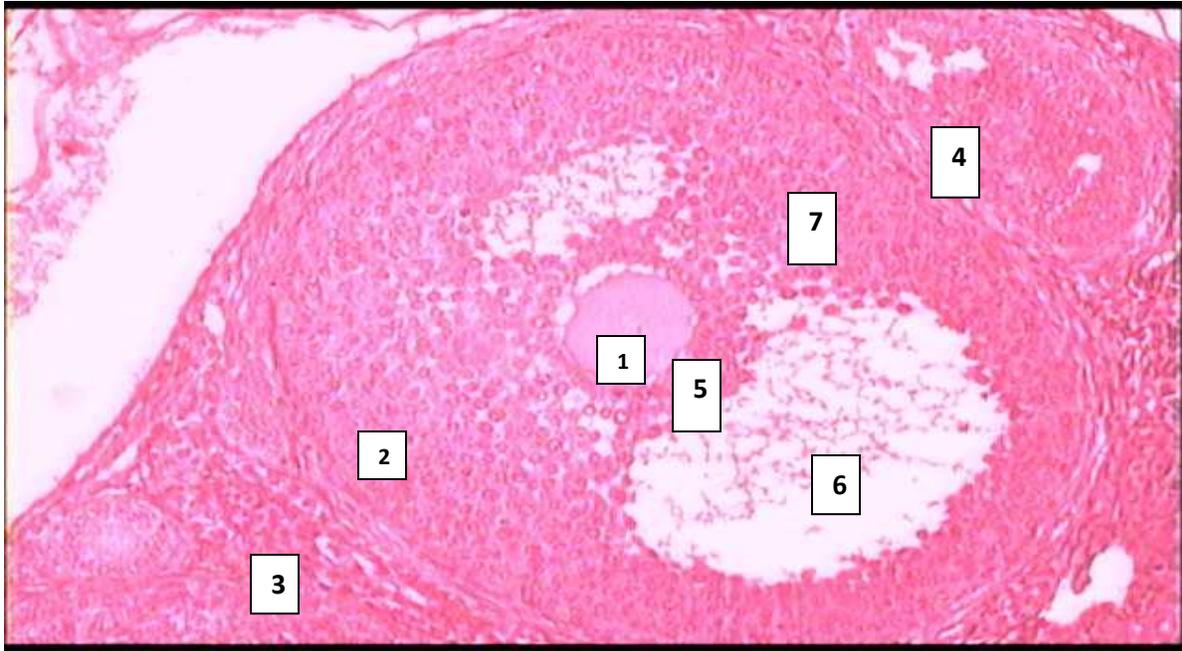


Figure 3. Tertiary follicles (1.oocyte, 2.granulosa cells, 3. Theca externa, 4.theca internal, 5. zona pellucida, 6.antral cavity, 7. cumulus oophorus) that found in the ovaries, (HE, 100 X)

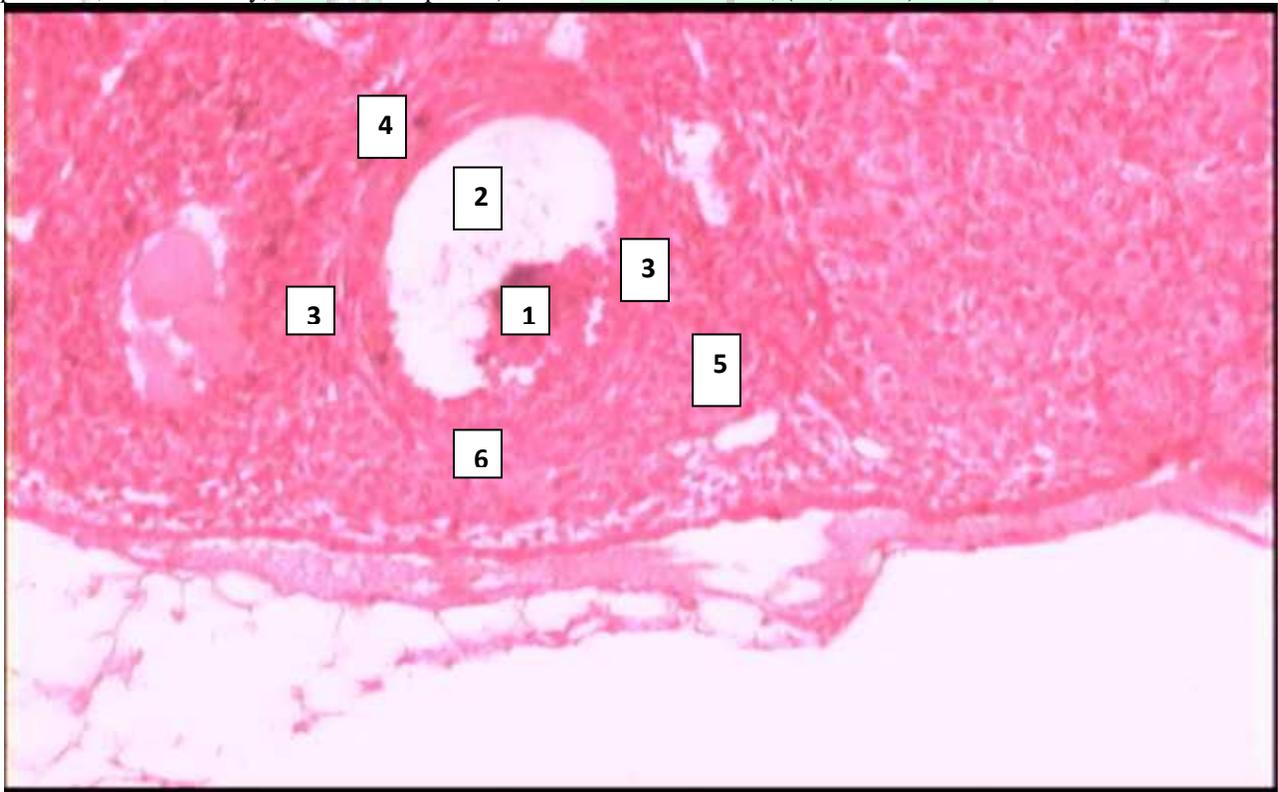


Figure 4. The follicle de Graaf (1.ovum, 2.antrum, 3.basal lamina, 4.granulose cells, 5.theca externa, 6.theca internal, 7.cumulus oophorus) that found in the ovaries,(HE 100 X)

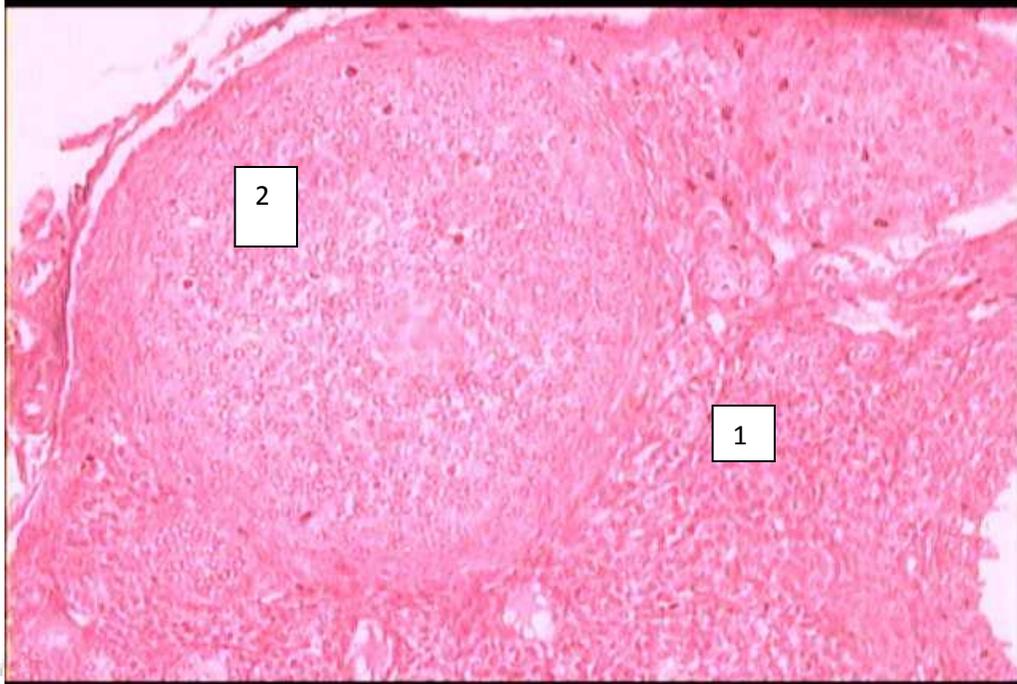


Figure 5. The corpus luteum (1.granulose 2.theca lutein cells and lutein cells) found in the ovaries, (HE, 100 X)

Discussion

Effect of Epinephrine against Primary Follicles: Based on the results of the study, administration of epinephrine will result in the number of primary follicles of mice decreased significantly compared with controls. Decrease in primary follicles is caused by the effects of epinephrine, which increased levels of epinephrine and decrease GnRH pulse. If there is excessive pulsation reduction, it can reduce and stop the secretion of FSH and LH. Decrease in FSH and LH will inhibit the growth of primary follicles.

This is in contrast with research Gusty and Reni (2006) which states that epinephrine cannot reduce the primary follicles in mice (*mus musculus*) Strain Japan. This is probably because of too small a dose of epinephrine (0.001 mg/20 g), not to affect the hypothalamus so as not to affect GnRH.

Effect of Epinephrine against Secondary Follicles: Epinephrine effect on the growth of secondary follicles resulting in a decrease in concentration of 0.004 mg / ml, 0.006 mg / ml, 0.008 mg / ml and 0.01 mg / ml (significantly different from controls) ; but not a decline in secondary follicles at a concentration of 0.002 mg / ml. In this study, secondary follicles have not experienced a decrease in konentration 0.002 mg / ml. This is because the administration of epinephrine at a dose of 0.002 mg / m, FSH not affects the production of secondary follicles. But, the production of FSH at a

concentration of 0.004 mg / ml to 0.01 mg / ml affected secondary follicles.

This is in contrast with research Gusty and Reni (2006) which states that epinephrine cannot lower secondary follicles in mice (*Mus musculus*) Strain Japan. This is due to the possibility of giving doses of epinephrine that are too small (0.001 mg/20 g), thus not affecting the formation of secondary follicles.

Effect of Epinephrine against Tertiary Follicles: the results showed a significantly different effect of epinephrine on tertiary follicles with a concentration of 0.004 mg / ml, 0.006 mg / ml, 0.008 mg / ml and 0.01 mg / ml in the control group. Because it affects the concentration of FSH in tertiary follicles, although did not differ at a concentration of 0.002 mg / ml, because this concentration of FSH do not affect the tertiary follicles.

Effect of Epinephrine against follicle de Graaf: From the survey results revealed the number of follicles de Graaf mice were not decreased despite the greater concentration of epinephrine is given. This is due to the FSH produced by the less so as not to affect the development of follicle de Graaf. This is in contrast with the results of Gusty and Reni (2006) which states that epinephrine can decrease de Graaf follicles in mice (*Mus musculus*) Strain Japan.

Effect of Epinephrine against the corpus luteum: based on the results of the study, administration of

epinephrine resulted in 1) a significantly decreased luteum corpus at concentrations 0.004 mg / ml, 0.006 mg / ml, 0.008 mg / ml and 0.01 mg / ml of LH who was affected due to the luteum corpus and does not decrease significantly at a concentration of 0.002 mg / ml of LH have not been affected due to end of the phrase.

Stress can activate the sympathetic nerves and adrenal response (Edward, 1993). Sympathetic nervous system activation by a stressor can cause local release of the neurotransmitter NE in postganglionic sympathetic nerve endings, while the stressor in adrenal medulla is to stimulate the release of epinephrine into the circulation (Norman, 1987). GnRH pulse control can be set by katekolaminergik (epinephrine and Norepinephrine) by giving positive feedback on GnRH secretion. Possibility of catecholamines is to change the frequency (and possibly amplitude) release of GnRH. The increase in GnRH pulse frequency and increased amplitude may decrease and stop the secretion of gonadotropin (GnRH Receptor Down Regulation) (Speroff 1994; Stefen, 2000).

The greater the stressor, the greater the stress response induced (Potter, 1997): (What does this phrase means? (Write again this phrase. We can't understand the signification)

Additionally stressors can also activate the sympathetic nervous system (even the release of Nor Epinephrine) and adrenal response (release of epinephrine) is excessive resulting in secondary amenorrhea. This can lead to an irreversible infertility.

Stressor is a powerful factor in influencing the gonadal axis. So that will affect the hypothalamus that inhibits gonadotropin, oxytocin and vasopressin (Laatikaine, 1991). If an increase in excessive stimulation or prolonged stress on the hypothalamus, adrenal cortex are not able to respond to increased secretion of ACTH by increasing cortisol spending, and even for to divert some cholesterol precursors into the androgen pathway. The result is an increase in DHEA, androgen excess does not inhibit ACTH but inhibits gonadotropin, which will reduce the secretion of FSH and LH that impact the disruption of oogenesis and inhibit ovulation in women (Sherwood, 2001).

Elevated levels of epinephrine and NE can degrade GnRH pulse. If this pulsation reduction can reduce and stop the excessive secretion of FSH and LH, decrease in FSH and LH will inhibit the growth of ovarian follicles and decrease the synthesis of estrogen and progesterone in the ovaries (Gosden, 2001).

Decreased synthesis of estrogen and progesterone may increase the reduction in the number of ovarian follicles. Normally GnRH is secreted in episodic pulsations. It is important for normal FSH and LH secretion (Ganong, 2001). In the present study can be shown that changes in FSH and LH secretion is

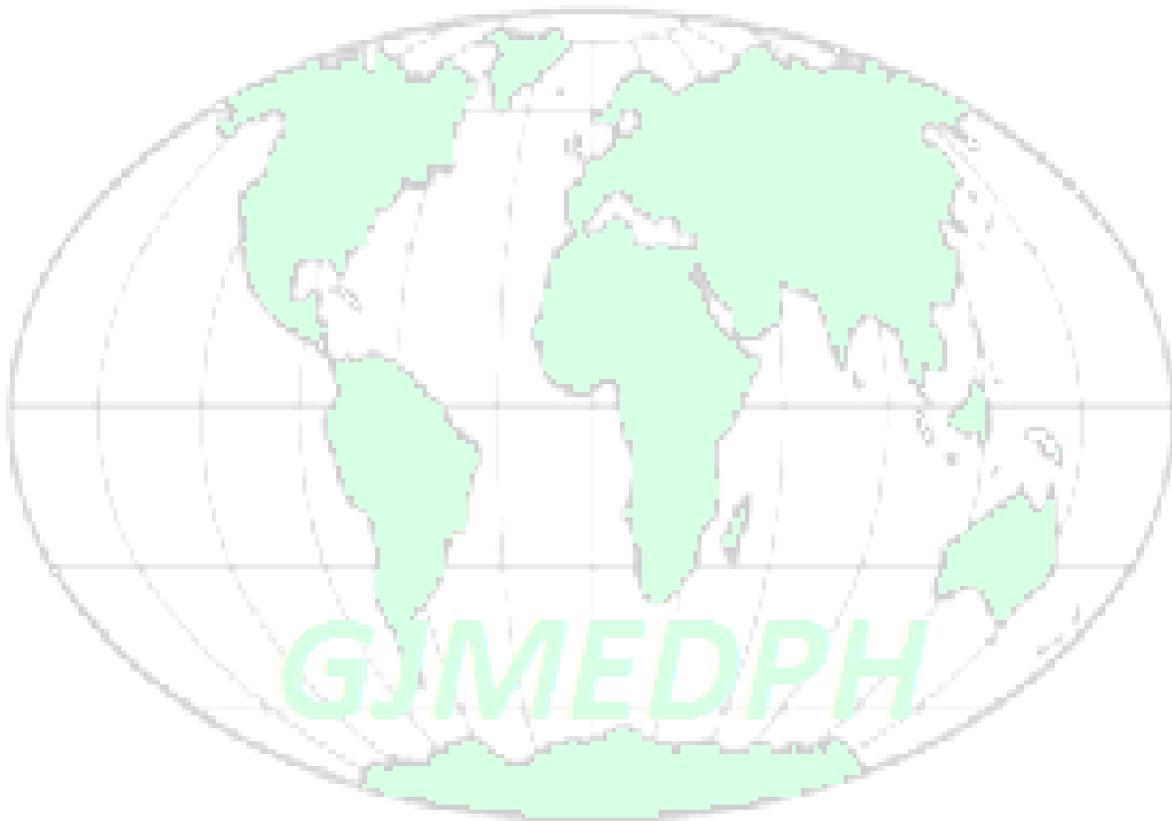
pulsatile GnRH requires expenditures by the frequency amplitude in the critical limit (Speroff, 1994).

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