

Increased Corticosterone Levels due to Chronic Stress Reduces IGF-II Expression in *Rattus norvegicus*

Ade Septiari Rahman, B.Mid¹, Reny I'tishom², Ashon Sa'adi³

¹Master Degree of Reproductive Health Science Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia,

²Lecturer in Department of Medical Biology Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia,

³Lecturer and Consultant in Department of Obstetrics and Gynecology Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

The optimal folliculogenesis process describes a good condition of the female reproductive system. One of the main growth factors in the folliculogenesis is IGF-II which is important for proliferation and differentiation of granulosa cells and also steroidogenesis. Chronic stress can cause ovulation failure and folliculogenesis disorders. Increased corticosterone levels due to chronic stress will cause disruption in the GnRH pulse, causing a decrease in the gonadotropin and lead to folliculogenesis disorders, and decrease IGF-II production. This study aims to prove the effect of increased corticosterone levels due to chronic stress on IGF-II expression in the ovaries of *Rattus norvegicus*. The research sample consisted of 34 *Rattus norvegicus* which were divided into 2 groups. The treatment group was given Chronic Unpredictable Mild Stress (CUMS) method for 20 days. Corticosterone levels were detected by the ELISA method as a marker of chronic stress and IGF-II expression was evaluated by immunohistochemical methods. The results of statistical tests showed that there were significant differences in corticosterone levels ($p = 0.000$) and IGF-II expression ($p = 0.042$) in the two groups ($p < 0.05$). So the conclusion is increased corticosterone levels due to chronic stress can reduce IGF-II expression in the ovaries of *Rattus norvegicus*.

Keywords: Corticosterone, Chronic Stress, CUMS, IGF-II, Folliculogenesis

Introduction

Infertility problems are experienced in women as much as 35% of all cases⁽¹⁾. According to WHO, the most common causes of infertility in women are ovarian failure, which is 33% was caused by folliculogenesis disorders⁽²⁾. Stress that that occurs continuously or chronic stress can cause ovulation failure and folliculogenesis disorders, and can subsequently lead to infertility.

Stress has been shown to inhibit oocyte maturation and produce many immature oocytes⁽³⁾, and reduce the number of dominant follicles⁽⁴⁾. Stress also causing a decrease in the number of fertilized oocytes, pregnancy rates, live births and birth weight in women undergoing in vitro fertilization (FIV)⁽⁵⁾. Chronic stress has also been shown to reduce the thickness of the antral follicular granulosa cells, and as a result, folliculogenesis is disrupted⁽⁶⁾.

Chronic stress can cause the distress condition, which causes the hypothalamus to produce Corticotrophin Releasing Hormone (CRH)⁽⁷⁾, and then the anterior pituitary will produce Adenocorticotrophine Hormone (ACTH) which stimulates the adrenal glands to produce glucocorticoid hormones, namely cortisol^(7,8). Glucocorticoid synthesis in response to chronic stress

Corresponding Author:

Dr. Ashon Sa'adi, MD, SpOG-K

Lecturer and Consultant

Department of Obstetrics and Gynecology Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital; Tambaksari, Surabaya, East Java, Indonesia

E-mail: ashon.saadii@fk.unair.ac.id

Phone: +628170121121

will further impair reproductive function^(7,9).

There are various kinds of growth factors that help the folliculogenesis process. One of them is the main and most abundant growth factor in the human ovarian system during folliculogenesis, namely Insulin-like Growth Factor-II (IGF-II). IGF-II plays a role in cell growth, proliferation, differentiation and acts as a regulator of the remodeling of the extracellular matrix of granulosa cells⁽¹⁰⁾. In addition, IGF-II also increases the production of estradiol and progesterone by granulosa cells⁽¹¹⁾.

Increased levels of cortisol (corticosterone in *rattus*) due to chronic stress can indirectly interfere with GnRH pulses, causing a decrease in the amount of gonadotropin hormones. Because gonadotropins stimulate IGF production, the IGF-II formed will be reduced^(7,9). Activation of IGF-II from the IGF-I and AKT receptors required FSH to stimulate CYP19A1 expression and granulosa cell proliferation. So with reduced IGF-II production, it will cause cell proliferation and steroidogenesis disrupted, followed by folliculogenesis disorder^(11,12).

This study aims to prove that an increase in the hormone corticosterone due to chronic stress can result in a decrease in IGF-II expression in *Rattus norvegicus*.

Material and Method

The design of this research is True Experimental with Post Test Only Control Group design. The study was conducted from April to June 2019 at the Animal Laboratory, Department of Biology, Faculty of Science and Technology, Airlangga University. The sample unit in this study was female rats (*Rattus norvegicus*) 5-6 months old who had given birth, weighing 300-350 g and in good health. All sample units were adapted for 7 days and injected PGF2 α at a dose of 25 μ g/gBW intraperitoneally to synchronize lust. This aims to get the diestrus phase at the time of surgery. Female rats were divided into 2 groups consisting of 17 individuals per group. The treatment was carried out using the Chronic Unpredictable Chronic Stress (CUMS) method, which is

a model of giving minor intensity stressors that resemble stressors of daily life for several weeks⁽¹³⁾. In this study, stress exposure was given for 20 days with 8 forms of treatment.

Ovarian tissue samples are taken surgically, and blood samples are taken intracardially. Corticosterone levels taken from the blood were detected by the ELISA method. IGF-II expression was detected by immunohistochemistry. The data obtained were then converted with the Remelle Scale Index. Statistical tests were performed using SPSS 25 software. The data obtained were first tested for normality by the Kolmogoroff-Smirnov and Shapiro-Wilk tests. Followed by a parametric statistical test, the Independent T-Test. If the data is not normally distributed, the Mann Whitney statistical test is used.

Results and Discussion

The sample unit in this study was 34 *Rattus norvegicus* which were divided into two groups. The control (K1) and treatment (K2) groups each consisted of 17 *Rattus norvegicus*. In the K1 group, there were two samples whose corticosterone level were undetectable, so they were excluded. In the K2 group, there was one sample unit that dropped out due to death. The death of *Rattus norvegicus* occurred on the 16th day of the stressor treatment. Thus, the final number of sample units for each group is 15 in K1 and 16 in K2. This number is qualify the minimum sample size in this study (15).

Table 1. Mean and SD of corticosterone levels in the blood serum of *Rattus norvegicus*

| Group | N | Mean \pm SD (ng/mL) |
|-------|----|-----------------------|
| K1 | 15 | 23.29 \pm 8.42 |
| K2 | 16 | 72.84 \pm 64.03 |

The mean of corticosterone levels in the K2 group (72.84 \pm 64.03) were higher than the K1 group (23.29 \pm 8.42).

Table 2. Mann Whitney analysis results of corticosterone levels in the blood serum of *Rattus norvegicus*

| P Value | Different tes analysis |
|---------|------------------------|
| 0,000* | Mann Whitney test |

*Significantly different $P < 0,05$

The results of the Mann Whitney test showed

$p = 0.000$ ($p < 0.05$), it means that there was a significant difference in corticosterone levels between the K1 and K2 groups.

The examination of IGF-II expression using immunohistochemical methods. Observation of IGF-II expression was carried out using a microscope connected to a computer with 64-bit Nikon NIS-Elements F-4.60.00 software. IGF-II expression can be seen in Figure 1.

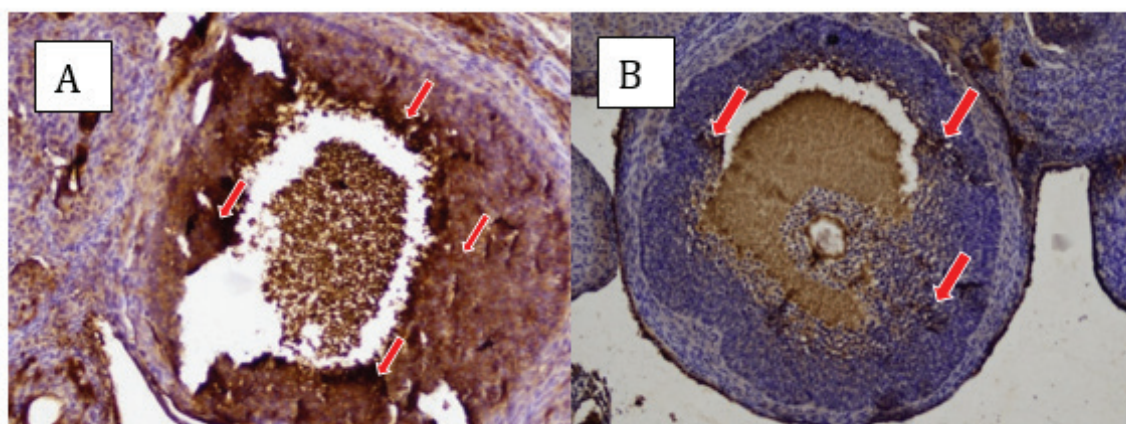


Figure 1. Comparison of IGF-II expression in ovarian tissue of *Rattus norvegicus*. Detected with IGF-II monoclonal antibody with Immunohistochemical method, 200x magnification. Cells expressing IGF-II are indicated by a brown appearance indicated by red arrows. (A) Expression of IGF-II in the K1 group (B) Expression of IGF-II in the K2 group.

Table 3. Mean and SD of IGF-II expression in the ovary of *Rattus norvegicus*

| Group | N | Mean ± SD (IRS) |
|-------|----|-----------------|
| K1 | 15 | 3.73 ± 1.79 |
| K2 | 16 | 2.44 ± 1.15 |

Based on the table, the mean of IGF-II expression in the K2 group was lower (2.44 ± 1.15) than the K1 group 3.73 ± 1.79 .

Table 4. Mann Whitney analysis results of IGF-II expression in the ovary of *Rattus norvegicus*

| P Value | Different tes analysis |
|---------|------------------------|
| 0,042* | Mann Whitney test |

*Significantly different $P < 0,05$

The results of the Mann Whitney test showed the value of $p = 0.042$ ($p < \alpha$), it can be concluded that there was a significant difference in IGF-II expression of K1 and K2 groups.

The results showed that after 20 days treatment of stress, corticosterone levels in the treatment (K2) group increased significantly compared to the control (K1) group. And after giving CUMS method for 20 days, the IGF-II expression in the treatment group was lower than the control group (significantly different). This proves that chronic stress, which is characterized by an increase in corticosterone levels, can result in a decrease in IGF-II expression. Increased cortisol levels due to chronic stress will disrupt the GnRH pulsation, causing a decrease in gonadotropins. Because gonadotropins stimulate IGF production, the IGF-II formed will be reduced^(7,9). The increased cortisol hormone will lower the FSH hormone levels. FSH is also a strong enhancer of IGF-II expression, so that by decreasing FSH due to increased cortisol, IGF-II will also decrease, and consequently cell proliferation can be disrupted, and steroidogenesis as well⁽¹¹⁾.

IGF-II interacts with IGF-1R, which causes activation of the PI3-kinase/Akt signaling pathway which impacts protein translation as well as cell proliferation and differentiation. IGF-II also interacts with IGF-2R which results in increasing steroidogenesis in human and mouse granulosa and theca cells. IGF-II increases the production of estradiol and progesterone by granulosa cells⁽¹⁴⁾. Studies on human ovarian follicle cultures proved that the addition of IGF-II to culture media stimulated primary follicle production and reduced the number of atretic follicles compared to the control group. This means that if IGF-II decreases, folliculogenesis can't be optimal because IGF-II serve as a protector and stimulator for follicular development⁽¹⁵⁾.

Conclusion

The increase in corticosterone levels as a result of chronic stress can reduce IGF-II expression in *Rattus*

norvegicus.

Ethical Clearance: Ethical clearance of this study was taken from Ethical Committee of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

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Conflict of Interest: None

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