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Elevated Corticosterone Level Due To Chronic Stress on Hb-Egf Expression as a Marker of Endometrial Receptivity Disorder in *Rattus norvegicus*

Risya Secha Primindari[1], Ashon Sa'adi[2], Reny I'tishom[3]

[1] Master degree of Reproductive Health Science, [2] Department of Obstetrics and Gynecology,
[3]Departemen of Biomedical Science, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

Abstract

Endometrial receptivity is a physiological state in which the endometrium obtains an adhesive phenotype that allows embryo implantation and have a role in the problem of implantation failure. Endometrial receptivity disorders can be caused by interference with the Hypothalamus-Pituitary-Gonad (HPG) axis due to the activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis by stress. HB-EGF is a biomarker of endometrial receptivity that plays a role in the decidualization of endometrial stromal cells to reach the receptive state and initiation of implantation. Corticosterone is the dominant glucocorticoid hormone in rodents, as is cortisol in humans. High corticosterone due to chronic stress triggers disruption of homeostasis in the endometrium by resulting in decreased levels of HB-EGF. This study aims to find out the effect of increased corticosterone levels due to chronic stress on HB-EGF expression in endometrium *Rattus norvegicus*. This research has obtained ethical eligibility from the Research Ethics Commission of the Faculty of Medicine Airlangga University. The samples on this study were 34 rat (*Rattus norvegicus*) which were divided into 2 groups, the control group and the stress treatment group using the Chronic Unpredictable Mild Stress (CUMS) method. Corticosterone level were obtain from blood serum detected via ELISA and HB-EGF expression was obtained from endometrial in diestrus phase was evaluated by immunohistochemical methods. Corticosterone levels in the stress treatment group were higher (72.84 ± 64.03) than in the control group (23.29 ± 8.42). HB-EGF expression in the stress treatment group was lower (82.06 ± 5.91) than in the control group (118.76 ± 13.20). Statistical tests showed significant differences in HB-EGF expression in endometrium Rattus norvegicus p = 0,000 (p <0.05). Elevated level of corticosterone can decrease HB-EGF expression in endometrium *Rattus norvegicus*.

Keywords : *chorticosterone, chronic stress, HB-EGF expression, endometrial receptivity*

Introduction

Endometrial receptivity is a physiological state in which the endometrium obtains an adhesive phenotype that allows embryo implantation and occurs over a period of time known as the implantation window in the middle phase of endometrial secretion. In this phase endometrium is very dependent on the presence of endogenous or exogenous progesterone and stimulation of 17β-estradiol. Implantation can only occur when the endometrium is in its receptive phase[1,2].

A study shows that endometrial receptivity has a 60% role in the incidence of implantation failure [3]. Another study conducted in India found that 25% of implantation failures are known to be caused by endometrial receptivity[4]. As many as 30% of secondary infertility in women is caused by implantation failure and endometrial receptivity disorders being one of them[5,6,7]. One factor that can interfere with endometrial receptivity is stress[8].

Endometrial receptivity disorders can be caused by several factors, such as interference with the

Corresponding Author:
Dr. Reny I'tishom, M.Sc; Tambaksari, Surabaya, East Java, Indonesia; ritishom@fk.unair.ac.id; +628121644432

Hypothalamus-Pituitary-Gonad (HPG) axis due to the activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis by stress. Excessive activity of Corticotrophine Releasing Hormone (CRH) in the hypothalamus, in response to stress, is a mechanism for suppression of GnRH. Furthermore, as the body's effort to adapt to stress causes the production of a glucocorticoid hormone, cortisol, by the adrenal glands. Increased cortisol levels can indirectly cause interference with GnRH pulsation, causing a decrease in the amount of gonadotropin hormone produced by the anterior pituitary[9]. Increased cortisol results in impaired levels of the hormones estrogen and progesterone in the ovary. Decreased levels of the hormones estrogen and progesterone in the ovary also have an impact on estrogen and progesterone levels in the endometrium[10].

One of the biomarkers that plays a role in determining endometrial receptivity is Heparine Binding Epidermal Growth Factor (HB-EGF) which affects cell-to-cell interactions and is a dependent estrogen progesterone[1]. HB-EGF is needed for normal decidualization of endometrial stromal cells to reach the receptive state in the endometrium and for the initiation of implantation. HB-EGF has been identified as an initial mediator of embryo-uterine interactions during implantation and is expressed both in the blastocyst and in the endometrium during implantation and plays a role in stimulating embryonic development during hatching. The high cortisol hormone due to chronic stress triggers homeostasis in the endometrium due to the inhibition of the formation of the hormone progesterone which results in decreased levels of HB-EGF[6]. When HB-EGF levels decrease, the number of mature ErbB4 and HB-EGF receptors released in the endometrium decreases. ErbB4 has an important role in stimulating blastocyte implantation. ErbB4 in endometrium will communicate in juxtacrine with ErbB1 contained in blastocytes. Communication between these two receptors is an important key in mediating implantation[10,11].

Chronic stress in this study uses the Chronic Unpredictable Mild Stress (CUMS) method. Chronic Unpredictable Mild Stress is giving various treatments as a stressor and resembles a stressor of everyday life that is not too heavy but continuously[12]. This method has been shown to significantly increase corticosterone levels (cortisol in mice) within 20 days[13]. In Rattus, cortisol secretion is replaced by corticosterone[14]. Chronic stress increases glucocorticoid synthesis and secretion (cortisol in humans and corticosterone in rodents). Increased glucocorticoids are biomarkers for stress and depression disorders[15]. Based on this background, this study was aim of to find the effect of increased corticosterone on HB-EGF expression as a marker of interference with endometrial receptivity.

Material and Method

This study was true laboratory experimental designs with randomized post test only control group design. The study was conducted from April to June 2019 at the Experimental Animal Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga. The samples of this study were female rats *(Rattus norvegicus)* Wistar strains aged 5-6 months, have ever given birth to the consideration of mice in fertile conditions, never been used as an object of research before, and in healthy condition. All animals are injected with PGF2α at a dose of 25 µg / g body weight intraperitoneally to synchronize the lust cycle. The purpose of synchronizing the lust cycle is to equalize and obtain the diestrus phase during surgery. Female rat was taken randomly into 2 groups, each group consisting 17 female rat. The control group (K1) consisting rat with negative treatment were mice that were not given a stressor and the treatment group (K2) consisting rat with stressors treatment. Stressors treatment in rat using Chronic Unpredictable Mild Stress (CUMS) method for 20 days.

Corticosterone level were obtain from blood serum detected via ELISA and HB-EGF expression was obtained from endometrial in diestrus phase was evaluated by immunohistochemical methods. HB-EGF was assessed semi-quantitatively on the scale of Rammele-Stegner using the Immuno Reactive Score (IRS). Statistical analysis uses IBM SPSS Statistic 25. Data with normal distribution was tested by Independent T test. If the data was not normally distributed, the data was tested by the Mann Whitney test. This study uses a significance level of 0.05 with a confidence level of 95%.

Findings

1. Corticosterone Levels

Table 1. The mean and standard deviation of corticosterone levels in serum *Rattus norvegicus*

Group	n	Corticosterone Levels
		Mean ± Standard Deviation
K1	15	23.29 ± 8.42
K2	16	72.84 ± 64.03

The results showed a mean of corticosterone levels in the treatment group was higher (72.84 ± 64.03) than control group (23.29 ± 8.42).

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

Group		P Value	Different test analysis
K1	K2	0,000*	Mann Whitney Test

*Significantly different $P<0,05$

Analysis of the Mann Whitney test showed that there were significant differences in the corticosterone levels between the control and treatment groups with a value of $P= 0,000$ ($P<0.05$).

2. HB-EGF Expressions

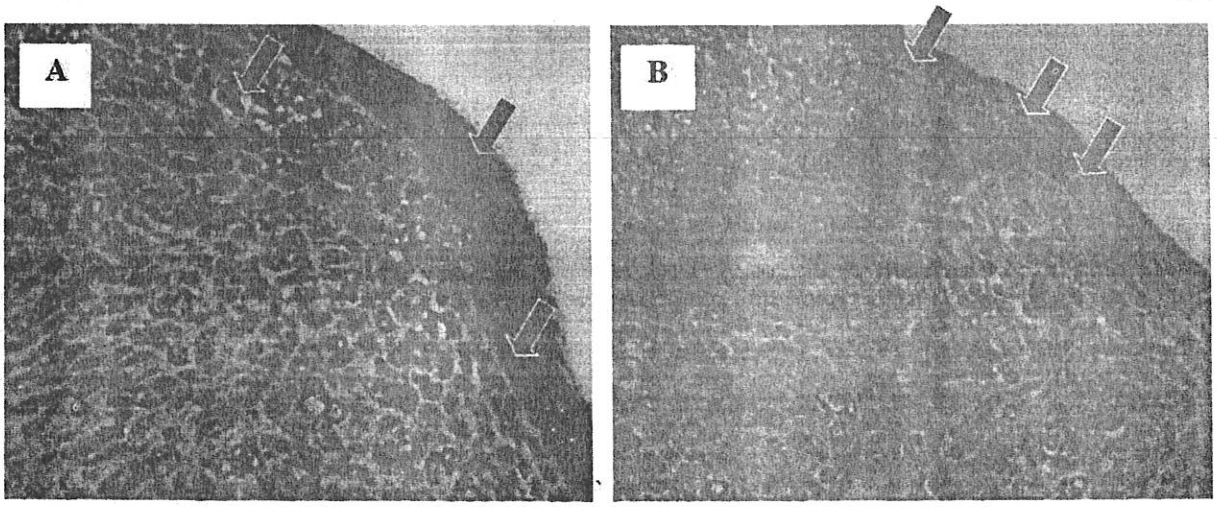

Figure 1. Comparison of HB-EGF expressions represented by chromogen brown color on the endometrial tissue, red arrows showing the maximum expression area using immunohistochemical staining, 400x magnification; Miconos microscope MCX50LED; Optilab Plus camera. (A) HB-EGF expression in the control group (B) HB-EGF expression in the treatment group.

Table 3. Mean and standard deviation of endometrium HBEGF expression

Group	n	HBEGF expression (IRS)
		Mean ± Standard Deviation
K1	15	118.76 ± 13.20
K2	16	82.068 ± 5.91

The examination results of the endometrium HBEGF expression showed a mean and deviation standard in the stress treatment group (82.068 ± 5.916) lower than the control group (118.76 ± 13.208).

Table 4. Results of Independent T analysis of endometrium HBEGF expression

Group		P Value	Different test analysis
K1	K2	0,000*	Independent T

*Significantly different $P<0,05$

The results of the Independent T test showed that there was a significant difference in the HBEGF expression between the control and treatment groups with a value of $P= 0,000$ ($p<0,05$).

Discussion

Chronic stress increases glucocorticoid synthesis and secretion (cortisol in humans and corticosterone in rodents). Increased glucocorticoids are biomarkers for stress and depression disorders[15]. Corticosterone is the dominant glucocorticoid hormone in rodents, as is cortisol in humans[14].

In normal conditions, glucocorticoids will provide negative feedback by suppressing CRH secretion to prevent excessive stress responses, but this does not occur in chronic stress where the activity of the HPA axis continues[13]. Increased of glucocorticoids also affects the HPO axis directly by releasing GnRH release from the hypothalamus and suppressing the release of the gonadotropin hormone from the pituitary which can interfere with various mechanisms that occur in the ovaries so that it can lead to reproductive disorders[9].

There are many theories that present the mechanism of chronic stress on changes in reproductive function. Apart from the activity of CRH and glucocorticoids, which will be discussed next with the effect on the gonadotropin hormone, the disturbed circadian cycle also becomes the mechanism used in this study. The sample unit in this study is Rattus norvegicus which is a nocturnal animal[16], while all treatment activities are carried out in the morning - afternoon so that it changes the circular cycle of Rattus norvegicus. Disorders of sleep patterns that are applied to experimental animals cause disruption of the circandian rhythm in the central arrangement of the circandian rhythm in the hypothalamus, the Supra Chiasmatic Nucleus (SCN). Circandian rhythm is the body's biological clock 24 hours a day. This system regulates hormone secretion, and one of the physiological processes of the sleep cycle is awake[17]. Disorders of sleep patterns that are not sought to overcome it will fall in a state of psychosocial stress. In addition to the physical stress that is given, the change in circular rhythm also has an impact on the psychological stress of the experimental animal, thereby increasing the existing stress. The circadian cycle is an integral part of the reproductive system, when the 24-hour program is irregular the endocrine system can be disrupted. The high corticosterone levels in Rattus norvegicus in this study are in line with the Lopez-Lopes *et al.* study which states that CUMS can increase corticosterone levels with a minimum of treatment for 20 days[13].

HB-EGF has been identified as an initial mediator of embryo-uterine interactions during implantation and is expressed both in the blastocyst and endometrium during implantation and is a dependent estrogen and progesterone[1,6]. Chronic stress causes elevated serum corticosterone levels, resulting in impaired estrogen and progesterone levels in the ovaries. Decreased levels of the hormones estrogen and progesterone in the ovary also have an impact on estrogen and progesterone levels in the endometrium[10]. Increased cortisol has an impact on homeostasis disruption in the endometrium resulting in decreased levels of HB-EGF which is a dependent estrogen and progesterone [6]. HB-EGF is expressed in luminal endometrial epithelial cells and on the surface of pinopods. Disregulation in HB-EGF expression in the endometrium has been associated with infertility whose causes cannot be explained (unexplained infertility). HB-EGF is needed for normal decidualization of endometrial stromal cells to reach the

receptive state in the endometrium and for the initiation of implantation[1,18].

HB-EGF performs two simultaneous functions during the human implantation as an attachment factor and a growth factor. It was reported that HB-EGF plays an important role in the preparation of the uterine luminal epithelium for blastocyst attachment at the beginning of pregnancy. HB-EGF, as a growth factor, accelerates the development of human embryos to the blastocyst stage and their subsequent hatching from the zona pellucida. HB-EGF, as an attachment factor, upregulates many important proteins expressed from a uterine luminal epithelial surface such as integrin β3, leukemia inhibitory factor (LIF), and HOXA10. Integrin αvβ3 serves for osteopontin to mediate the embryo attachment. LIF stimulates human embryo development to the blastocyst stage and is required for embryo implantation. HOXA10 was found in the human endometrium during the mid-secretory phase abundantly and may be involved in implantation and decidualization of endometriumduring early pregnancy. HOXA10 induction by progesterone during the window of implantation leads to a blockage in the stromal cell cycle, facilitating decidualization[19].

HB-EGF induces endometrial cell proliferation through activating the cascade of ERK1 / 2 signals in epithelial cells and increasing DNA synthesis and cyclin D3 in stroma cells[10,20]. Decreased levels of HB-EGF have an impact on disruption of endometrial proliferation and angiogenesis, this can have an impact on the inability of the endometrium to reach its receptive phase. The sequence of endometrial proliferation and angiogenesis disorders can be interpreted as a disturbance in endometrial receptivity.

Conclusion

Elevated level of corticosterone can decrease HB-EGF expression in endometrium *Rattus norvegicus*.

Ethical Clearance: Ethical clearance of this study was taken from Ethical Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. Number: 129/EC/KEPK/FKUA/2019

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Conflict of Interest: The authors have no conflicts of interest.

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