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Research Article

THE EFFECT OF PSYCHOLOGICAL STRESS ON MPF INTRAFOLLICULAR

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ABSTRACT

Objective: The aim of the study is to investigate the influence of cortisol and Heat Shock Protein 70 (HSP70) on the stressed mice to maturation promoting factor (MPF) expression intrafollicular.

Methods: Experimental laboratory with Randomized Post Test Only Control Group Design was carried out on intrafollicular mice. Divided into two groups, experimental and control groups. The experimental group was given a 95 dB 4 hours/day noisy exposure for 5 days which was analogous to psychological stress in humans and the control group was not given noisy exposure. Furthermore, both groups were examined for cortisol levels to ensure stress in mice. Heat shock protein 70 (HSP70) expression was examined as the main regulatory protein for stress response and Maturation promoting factor (MPF) expression which is a mediator for oocyte maturation.

Results: Psychological stress by 95 dB/4 hours/day noisy exposure for 5 days significantly increased serum cortisol levels in experimental group (p=0.000) and HSP70 expression (p=0.000). The effect of Increased cortisol levels and HSP70 expression significantly decrease in MPF expression (p=0.000).

Conclusion: The study concluded that psychological stress could be seen by increasing cortisol and HSP70 expression affected to decreasing MPF expression intrafollicular

Keywords: Stress, Cortisol, Heat Shock Protein 70, Maturation promoting factor.

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INTRODUCTION

Oocyte maturation is defined as the starting and confirmation of the first meiotic division, it is start from the vesicular germinal stage to metaphase II stage, followed by cytoplasmic maturation which is crucial for the fertilization and early embryonic development process. The Nucleus maturation related to meiotic division until the metaphase II stage, whereas cytoplasmic maturation related to preparation of oocyte cytoplasm for embryo fertilization and development [5]. Oocytes are unique cell because they have meiotic phase, this phase only occurs in the reproductive phase. Oocytes need the ability to reinvent the first meiotic division towards MII and the cytoplasmic changes needed for embryo fertilization and development [20]. Cellular maturation process is accompanied by specific protein expression. The protein activity is modulated by specific kinases and phosphatases that control cellular processes such as cell growth, differentiation, cell cycle and meiotic. During the maturation of meiotic oocytes, the activation of MPF and MAPK as protein kinase plays a dominant role. MPF must be activated so that the cell can transit from G2 to M phase [15].

Maturation promoting factor (MPF) is the cyclin-Cdk complex that stimulates the mitotic and meiotic phases of the cell cycle. MPF promotes the entrance into mitosis (the M phase) from the G2 phase by phosphorylating multiple proteins needed during mitosis. MPF is activated at the end of G2 by a phosphatase, which removes an inhibitory phosphate group added earlier. The MPF is also called the M phase kinase because of its ability to phosphorylate target proteins at a specific point in the cell cycle and thus control their ability to function [21,23]. MPF activity induces the process of mitosis in somatic cells and miosis in oocytes. Periodic activity is a typical of MPF. Each cell division requires a new fused MPF in somatic cells, whereas MPF degradation is needed at the end of mitosis so that the cell can complete mitosis [9].

Stress is a process stimulus activate the entire system and produces an organic response generating negative effects on health. The hormone mainly produced during stress is the cortisol which is secreted by the upper part of the adrenal gland, being an useful indicator as a biomarker to detect stress. In addition, cortisol plays an important role during the catabolic phase and has a negative effect on some metabolism. Under normal circumstances, stress hormones are released in small amounts throughout the day, but when faced with stress these hormone levels increase dramatically [11,12].

Stress affects many cellular processes which in turn cause physiological and immunological responses [8]. Cells can develop an efficient stress response by activating a protein control system (i.e., gene transcription, protein expression, and enzyme activity) or proceeding to a cell death signaling pathway to overcome the environment [16]. The response of cell stress is controlled by a complex molecular regulation system that is still not fully known [22].

On molecular level, a group of stress proteins called heat shock proteins (HSPs) generally increase synthesis. HSPs are highly conserved molecular chaperones that regulate folding/unfolding of proteins, as well as the assembly/disassembly of protein complexes to maintain normal housekeeping functions [2]. HSP expression levels are very low under physiological conditions, but they are quickly synthesized in response to various stimuli such as cold and heat stress, oxidative stress and severe mental toxicity, and increase cell voltage tolerance, maintain normal metabolism and increase their viability [6,17].

The molecular mechanism of how psychological stress affects MPF activation in oocytes is discussed in this paper.

MATERIALS AND METHODS

Animal

Mus musculus mice were used in the present study. All the mice were kept under controlled conditions of light and environmental and had free access to food and water. The estrus period of mice was confirmed at the same phase at the start of the study. The results of vaginal smear using hematoxylin-eosin (HE) staining. The study protocols were approved by the Institutional Animal Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya Indonesia (Ethical Clearence No 2.KE.067.04.2018) and animals care were carried out in compliance with the Committee guidelines for the purpose of control and supervision of animals experiments.

Psychological stress

Psychological stress exposure by 95 dB noise. TrueRTA software (real time audio spectrum analyzer) is used to produce noise. The noise intensity is measured by a real time sound analyzer (TES 1358) every day before animal exposure, by placing the analyzer in an animal cage in several locations, and taking the average from different readings. Animals exposed to noise 95 dB for 4 hours per day for 5 days. Control animals are placed in a cage (42 cm x 26 cm x 15 cm) located in a space of 1 m x 1 m x 2 m for the same period of time, but without noise.

Marker assays

Cortisol level is examined by ELISA, HSP70 expression by immunohistochemical method using Santacruz USA monoclonal antibody and MPF expression by immunohistochemical methods using Bioss USA monoclonal antibodies.

Data analysis

Data were analyzed by multivariate analysis. The Normality by Shapiro-Wilk test and the homogeneity by Levene test. Correlation between variables with normal and non-normal distribution was assessed using Independent sample t-test and Mann Whitney test. The effect of a variable on other variables was assessed using path analysis in SPSS.

RESULTS AND DISCUSSION

Based on the statistical results, psychological stress significantly increased serum cortisol levels in experimental group (p=0.000) and also significantly increased HSP70 expression (p=0.000). Increasing cortisol levels and HSP70 expression significantly caused a decrease in MPF expression (p=0.000). The comparison of variables are shown in Table 1.

Table 1: Different test of cortisol levels, HSP70 expression, and MPF expression

Variable	Group	N	Mean±SD	p
Cortisol	Control	10	6.98±1.14	0.000*
	Treatment	10	10.4±1.02	
HSP70	Control	10	7.16±0.6	0.000*
	Treatment	10	11.45±0.72	
MPF	Control	10	6.16±1.68	0.000*
	Treatment	10	2.11±0.85	

HSP70: Heat shock protein 70, MPF: Maturation-promoting factor

Assay 1: Effect of psychological stress on cortisol

Stress is a global threat that caused by various factors. The evidence that is quite widely expressed in the last decade focuses on the neurochemical, biochemical, and molecular effects that caused by stress in the Central Nervous System, the endocrine system, and the immune system [1]. Stress does not only occur at the level of the organism, but also at the level of organs and cells. Stress is a form of body reaction that determines survival. When the body is exposed to a condition that is considered threatening (stressor) by the cerebral cortex, a response will occur to deal with it. The stress response is closely related to two systems in the body, sympathetic-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenocortical (HPA) axis which can cause physiological changes in the body [19].

Psychological stress increased glucocorticoid levels through activation of the hypothalamic-pituitary-adrenal (HPA) axis [25]. Axis HPA disregulation on distress condition depends on prolonged stressors. This is causes the secretion of stress hormones to occur continuously [25]. Therefore, we analyzed cortisol levels as a marker of stress change.

The analysis of cortisol levels in control group and experimental group were 6.98±1.14 and 10.4±1.02. This showed a significant increase in cortisol levels (p=0.000). So, these results explain that stressor induced cortisol levels. It is clearly support that psychological stress affects the biological response by increased cortisol serum. Cortisol is a glucocorticoid hormone that plays an important role in various processes including metabolism, blood pressure, and regulation of immune responses. Cortisol has also been shown to have a biological correlation to several adverse health events. Therefore cortisol is an effective stress hormone that has a negative impact on oocyte maturation. In fact, several studies have shown that high cortisol levels will reduce the competency of oocytes, oocytes has an experience potential developmental disorders by triggering ovarian cell apoptosis [11].

Assay 2: Effect of cortisol level on HSP70 expression

Heat shock proteins (HSP) also play an important role in stress response. This is in accordance with the results of intra-oocyte HSP70 examination which shows that in psychological stress conditions higher expression of HSP70 is seen. Heat shock protein is a group of proteins whose expression increases when cells are exposed to high temperatures or other stresses. Heat shock protein is also known as a stress protein present in all cells that make up 5-10% of the total protein content in normal growth conditions, and can significantly induce up to 15% of total cellular protein content. The high production of heat shock protein can also be triggered by exposure to various types of environmental stress conditions, such as infection, inflammation, cell exposure to poisons (ethanol, arsenic, trace metals and ultraviolet light etc.) Hunger, hypoxia, nitrogen deficiency (in plants), or seizure of water. Under non-stressful condition, HSP carry the old proteins to the cells "recycling bin" and also help in proper folding to newly synthesized proteins and thus act as 'monitor' of the cellular proteins [14].

The path analysis showed effect of cortisol on HSP70 (Beta=0.767; p=0,000). The effect of cortisol on HSP70 occurs due to stressors exposure causes biochemical and physiological changes that mediated by the neuroendocrine system and characterized by an increase in cortisol. After cortisol binding, intracellular glucocorticoid receptors (GR) can exert their activity on target gene expression by transcription factors that depend on ligands (Celi *et al.*, 2012).

The immunohistochemical results of HSP70 expression in each study group are showed in the Fig. 1 below.

The Fig. 2 showed the experimental group has stronger chromagen brown color. It is clearly support the data analysis that there is an increasing HSP70 expression in experimental group and showed that highly level cortisol also induced HSP70 expression.

Heat shock protein 70 is powerful chaperones whose expression is induced in response to a wide variety of physiological and environmental insults. These proteins have different functions depending on their intracellular or extracellular location. Intracellular HSPs have a protective function. They allow the cells to survive potentially lethal conditions. The cytoprotective functions of HSPs can largely be explained by their anti-apoptotic properties. HSP70 can directly interact with different proteins of the tightly regulated programmed

cell death machinery and thereby block the apoptotic process at distinct key points. In contrast to intracellular HSPs, extracellularly located or membrane-bound HSPs mediate immunological functions. They can elicit an immune response providing a link between innate and adaptive immune systems.

In this study, increasing cortisol level on experimental group also increased hsp70 expression, so we predict that increasing HSP70 expression would protect the oocyte maturation process from stressors exposure.

Assay 3: Effect of cortisol level and HSP70 expression on MPF

Oocyte maturation is a period of critical development for obtaining competence into the embryonic stage after fertilization [4]. Many factors are present in the oocyte microenvironment which greatly influence the expression of several proteins, which in turn causes functional changes needed for oocyte maturation [22]. Oocyte maturation is characterized by two peaks of high maturation promoting factor (MPF) activity. The first occurs at the time of continuation of meiotic division and secondly, occurs during the termination of meiosis in stage MII [7,13]. During the interval between these two peaks, MPF activity is maintained at a fairly high level. Although the importance of MPF in oocytes is supported by a lot of data, it turns out that little is known about its specific molecular targets. But MPF is thought to be involved in several important stages of cell division, namely the separation of the nucleus, chromosome condensation, cytoskeleton rearrangement and cessation of transcription activity [9, 21].

Oocyte maturation is induced by sequential action of three substances: gonadotropic hormone (GTH) secreted from the pituitary gland, maturation inducing hormone (MIH) which is synthesized and secreted from follicular cells around the oocyte, and maturation promoting factor (MPF) which is produced and activated in the oocyte cytoplasm.

Our result study showed decreasing expression of MPF in the psychological stress group (experimental group), it is clearly support the data analysis that highly cortisol levels and HSP70 expressions affect to decreasing MPF expression (Beta=0.769; p=0.000). This supports some of the results study that the quality of oocytes and reproductive results is affected by stress. Stress can increase cortisol production, these changes directly or indirectly affect ovarian physiology [14]. Stress can cause inhibition of estradiol which is characterized by impaired granulosa cell function and the low number of oocytes harvested in assisted reproductive technology [4].

The immunohistochemical results of MPF expression in each study group are showed in the Fig. 3 below.

The Fig. 4 showed the control group has stronger chromagen brown color. It is clearly support the data analysis that there is a decrease MPF expression in experimental group.

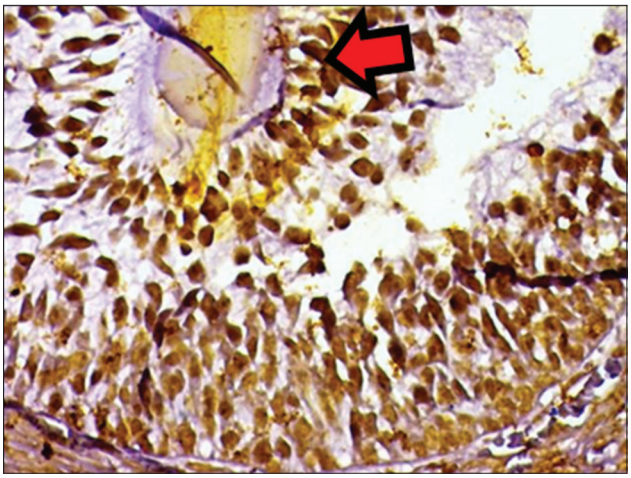

Fig. 1: Immunohistochemical results for heat shock protein 70 (HSP70) intrafollicular in the control group. The chromagen showed the cells that expressed HSP70

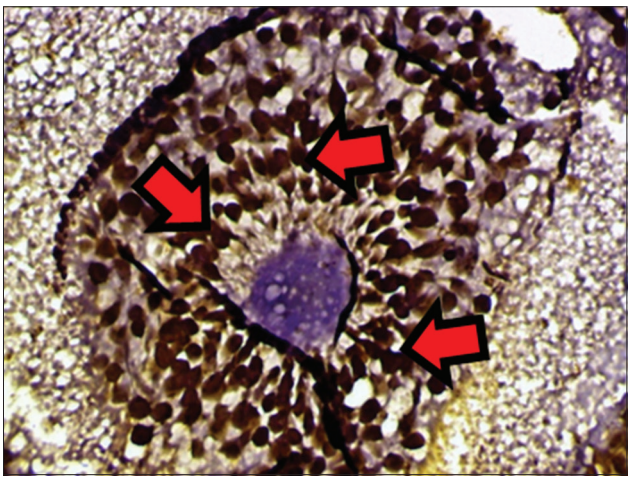

Fig. 2: Immunohistochemical results for heat shock protein 70 (HSP70) intrafollicular in the experimental group. The chromagen showed the cells that expressed HSP70

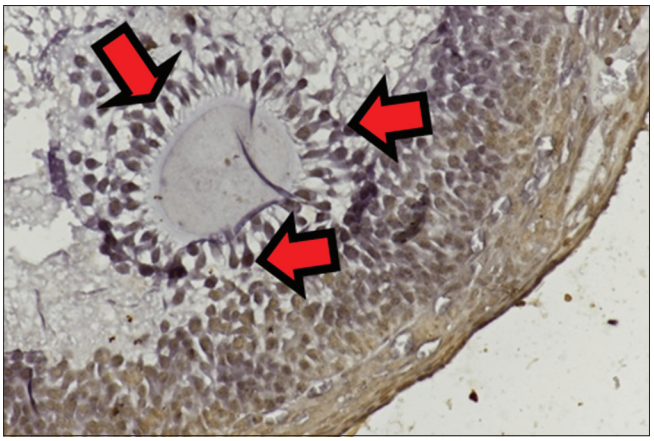

Fig. 3: Immunohistochemical results for maturation-promoting factor (MPF) in the control group. The chromagen showed the cells that expressed MPF

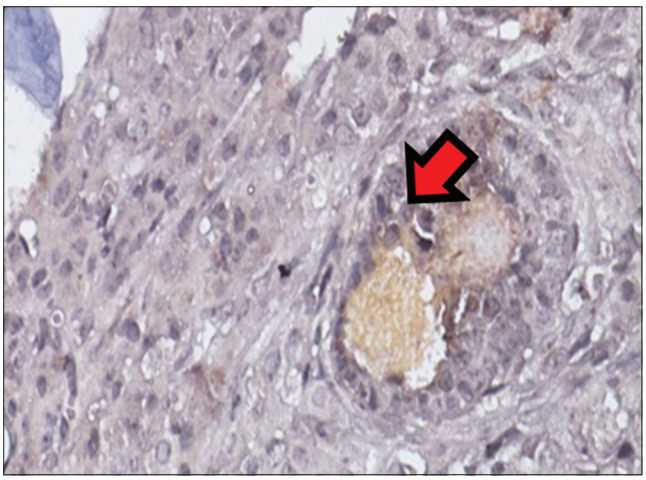

Fig. 4: Immunohistochemical results for maturation-promoting factor (MPF) in the experimental group. The chromagen showed the cells that expressed MPF

Oocyte maturation involves activation of various signal transduction pathways that activate MPF, so that decreased MPF expression can interfere with the oocyte maturation process. MPF activity induces the process of mitosis in somatic cells and miosis in oocytes. MPF induces the breakdown of the nuclear envelope (nuclear envelope breakdown/NEB) in oocytes because MPF can phosphorylate: (i) condensates, a large protein complex needed to twist DNA during mitosis; and (ii) several protein components of the nuclear membrane, including nucleoporin, a complex component of the nucleus pore where phosphorylation is one of the first steps in NEB [3,9,10].

Chaperone and heat-shock proteins are recognized as increasingly important proteins in the event of cell signaling because of their association with cell cycle components, regulatory proteins and mitogenic signal cascade members. If HSP70 plays its normal role as a chaperone protein, the thing that might happen is HSP70 will protect cells from stress because in normal cells, the HSP70 ATPase cycle performs several fundamental functions: (1) together with co-chaperones, HSP70 forms a protein folding mechanism and provides protein transportation into organelles; (2) assisted by HSP40, HSP70 recognizes irreversibly damaged proteins and, assisted by CHIP, Bag-1 and HSJ1 ubiquitinates these proteins, thereby targeting them for degradation via proteasomes; and (3) together with the co-chaperones HSP90, HSP40, Hip, Hop and Bag-1, HSP70 recognizes normal proteins containing the marker sequence KFPRQ and sends these proteins for degradation in lysosomes. Thus, the HSP70 ATPase cycle forms a protein quality control system or the Folding Refolding Degradation machinery (FORD) and, depending on the state of protein, sends the protein either for re-folding or for degradation. Because of the FORD machinery, a cell maintains protein homeostasis. The HSP70 ATPase cycle also controls the activity of key signalling proteins by maintaining these proteins in an inactive or active state by regulating their levels and by intracellular transport [18].

Heat shock protein-70 is implicated in the mechanism of cell reaction to a variety of cytotoxic factors. The protective function of Hsp70 is related to its ability to promote folding of nascent polypeptides and to remove denatured proteins. Many types of cancer cells contain high amounts of Hsp70, whose protective capacity may pose a problem for therapy in oncology. Hsp70 was shown to be expressed on the surface of cancer cells and to participate in the presentation of tumor antigens to immune cells. Therefore, the chaperone activity of Hsp70 is an important factor that should be taken into consideration in cancer therapy. The protective role of Hsp70 is also evident in neuropathology. Many neurodegenerative processes are associated with the accumulation of insoluble aggregates of misfolded proteins in neural cells. These aggregates hamper intracellular transport, inhibit metabolism, and activate apoptosis through diverse pathways. The increase of Hsp70 content results in the reduction of aggregate size and number and ultimately enhances cell viability [24].

CONCLUSION

The results of the study concluded that psychological stress affect to decreasing MPF expression and increasing HSP70 expression could not protect MPF activation, so Based on the results of this study, psychological stress inhibits the cytosolic feedback system of glucocorticoid receptor levels in the hypothalamic paraventricular. In this condition, the synthesis of pro-inflammatory cytokines is thought to be excessive so that it also impacts on the synthesis of other proteins including HSP70. But increasing HSP70 which should have a protective role, in this study HSP70 did not carry out its protective role. There are two possibilities, the first is the occurrence of HSP70 overexpression which has a negative impact on the expression of other proteins in the follicle. The second possibility is that the quantity of HSP70 has increased, but the quality of increasing HSP70 is not enough to carry out its protective role.

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