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The Effects of Unpredictable Stress on the LHR Expression and Reproductive Functions in Mouse Models

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Abstract : The objective of this study was to investigate the effect of chronic unpredictable stress on the reproductive function and ovarian luteinizing hormone receptor (LHR) expression. 9-week-old C57BL/6 female mice were randomly divided into two groups: control group and stressed group. Mice have been stressed twice a day for 35 days with 12 different stressors which were randomly selected. The results demonstrate that there is significant increase in the anxiety-related behaviors ($P < 0.05$), decrease body weight gain rate ($P < 0.01$) and decrease in the average of litter size in stressed mice compared with control group ($P < 0.01$). Furthermore, the rate of primary, secondary and early antral follicles in stressed mice significantly decreased ($P < 0.05$), whereas that of atretic follicles significantly increased compared with control mice ($P < 0.01$). The immunohistochemical analysis revealed that reduced LHR expression in granulosa cells of follicle and luteal cells of corpus luteum in response to chronic unpredictable stress. The western blot analysis revealed significantly decrease in LHR expression in the stressed mice ovaries compared with the control ($P < 0.05$). These results suggest that ovarian LHR expression affected by chronic unpredictable stress and the modulated ovarian LHR is responsible for ovarian follicular maldevelopment and reproductive dysfunction.

Key words **:** chronic unpredictable stress, follicular development, luteinizing hormone receptor, stress-related behaviors.

Introduction

Many previous studies showed that chronic psychosocial stress disrupts female reproductive and ovarian function (10, 31,33,40). Chronic stress on ovarian function has a suppressive effect mainly as suppression of gonadotropin secretion through the effects of hypercortisolemia on the hypothalamus and/or anterior pituitary gland (5,19,22). The release of glucocorticoids is a typical endocrine response to stress. The glucocorticoids are produced in the adrenal cortex when adrenocorticotropic hormone stimulates gluconeogenesis, and they provide energy for the stress response ("flight or fight"). Glucocorticoids work in response to stress by combining both stimulating and suppressive effects for ultimate self preservation. As a result, these steroids are responsible for many functions throughout the body, including important functions for fertility. During the stress response, resources are reallocated to prioritize self survival by suppressing reproductive axis. Many studies on couples having children suggest that chronic stress can cause reproductive malfunction. Various stressing factors, including malnutrition, anxiety, depression and infection induce an increase in glucocorticoids that repress reproductive functions through the hypothalamic-pituitary-gonadal (HPG) axis (11,29). In the hypothalamus, increased glucocorticoids suppress the secretion of gonadotropin-releasing hormone (GnRH) (6,14).

Although the effect of glucocorticoids in the pituitary is only incidental to its effect on secretion of GnRH, it decreases circulating level of luteinizing hormone (LH) (7). In females, LH supports theca cells in the ovaries which are supported by LH, produce hormonal precursors of estradiol synthesis and androgens. LH is also crucial for developmental progression during specific preantral follicle stage (44).

The luteinizing hormone receptor (LHR) acts a critical role in the control of reproductive functions such as ovulation, steroidogenesis in the ovaries, and testosterone synthesis by the Leydig cells of testis (3,15,26). LHR is able to bind both human chorionic gonadotropin (hCG) and LH with strong affinity. Thus it is nominated as LH/hCG receptor. LHR is also expressed in plenty of extra gonadal tissues (30) with several potential pathophysiological and physiological symptoms (1,2). Expression of LHR goes through compelling changes over the normal ovarian cycle. Antral follicles also

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express a small quantity of LHR that is almost limited to theca interstitial cells. Levels of LHR expression rise with follicle development in response to various paracrine factors reaching highest levels before ovulation happens (26,48). During the differentiation of estrogen, LHR expression is briefly decreased in response to preovulatory LH surge producing granulosa cells to luteal cells (20,24,25). Meanwhile, the differentiating granulosa cells remain refractory to LH on account of desensitization of the G protein coupled receptors (4,18). During mid-luteal phase, LHR expression reaches the maximum with elevated progesterone production. Next, the receptor levels drop upon the degeneration of the corpus luteum (20,24,25). LHR expression seems to be regulated by two ways: transcriptional and more seemingly, through post-transcriptional mechanisms.

As in the previous study, chronic unpredictable stress triggers a rise in glucocorticoids along the HPG axis. Increased glucocorticoids inhibit release of the GnRH with the result that circulating level of gonadotropin (FSH and LH) has declined. We suggested that ovarian LHR expression could be disturbed by chronic unpredictable stress, and the altered LHR is responsible for decreased ovarian growth and development. In the present study, we observe LHR expression and oocytes development in chronic unpredictable stress model to bring about psychosocial stress in mice.

Materials and Methods

Animals

C57BL/6 female mice were purchased from Daehan Biolink (Cheongju, Korea). The experiments were performed using 9-week-old C57BL/6 female mice weighing 15-20 g, and were conducted in accordance with the Kyungpook National University Guide for the Care and Use of Laboratory Animals.

Mouse stressed model

Tolerance can be increased when mice were repeatedly exposed to some predictable stressors. Therefore we assume that mice are exposed to unpredictable stress to eliminate unwanted tolerance. A classic stressed model was caused by chronic unpredictable stress (12,42,43,45). Mice were stressed twice daily for 35 days. Stressors consist of (1) damp bedding for 12 hours to 14 hours, (2) 45° cage tilting for 14 hours to 18 hours, (3) continuous light on (overnight), (4) water and food derivation for 24 hours, (5) strong level shaking for 10 minutes, (6) confinement in a tube for 2 hours, (7) no bedding (overnight), (8) 8-10°C cold water swimming for 3 minutes, (9) 4-5°C cold environment for 1 hour, (10) lights off for 3 hours (10:00 AM-1:00 PM), (11) 24 hours social isolation (1/cage), (12) 45°C oven for 5 minutes. The different stressors were randomly given to mice.

Open-field test

The open-field analysis was used to measure the anxious behavior of the mice in a strange environment. The 9-week-old C57BL/6 female mice (n = 5 for each group) were placed individually in a bright square box (26 cm × 26 cm, 250 lux). Parameters, including the spending time in center, moving distance in center, number of entries into the center, total moving distance were determined over 30 minutes using the TruScan Photo Beam Activity System (Coulbourn Instruments, Whitehall, PA, USA).

Marble-burying test

The marble-burying behavior tests were conducted using C57BL/6 mice. The mice were placed individually in plastic cages ($26 \times 20 \times 13$ cm^3) for 30 minutes. Twelve clean, light reflecting glass marbles (10 mm in diameter) were equally spaced 3-5 cm apart on 5 cm deep sawdust in the cages. Mice were put into these cages without water and food. The indicators of marble-burying behavior were presented as the number of marbles, at least, two-thirds buried within 30 minutes.

Fertility test

Mice were divided into control group (n = 4) and stressed group (n = 4). Stressed group was previously stressed twice daily for 35 days. After turn off the light, an intact male mouse was put in the each cage. Next morning, we identified vaginal plug and take a male mouse out from the cage. Pregnant female mice were stressed once daily during the period of pregnancy. After birth, we counted the litter size and checked the body weight of dams on post natal day 1 (PND1).

Tissue preparation

Under ether anesthesia, mice were sacrificed in the 36th day by exsanguination and their ovaries were rapidly dissected. The ovaries for observation of follicular morphology were fixed in 4% paraformaldehyde at room temperature for 48 hours, then dehydrated and embedded in paraffin. Paraffin sections (5 μm thick) were cut using a microtome (Leica Microsystems, Wetzlar, Germany) and then sections were stained with hematoxylin and eosin (H&E) or used in immunohistochemical analysis. The samples of western blot analysis were soaked in liquid nitrogen and stored at −80°C.

Ovarian follicular morphology

The paraffin-embedded ovaries were cut into 5 μm sections and stained with H&E. Follicles were counted when the nucleus of the oocyte was appeared. The numbers of primary, secondary, early antral, preovulatory antral, atretic follicles and corpus lutea were counted in every 10th section, and the total multiplied by ten to give the estimated number of follicles in the whole ovary. Then, the results were converted into a percentage. The follicles were classified according to the Myers *et al* (27): Primary follicles have a single layer of cuboidal granulosa cells. Secondary follicles possessed more than one layer of granulosa cells but antrum was not formed. Early antral follicles possessed multiple layer of granulosa cells and one or two small spaces of antrum. Preovulatory follicles had a border of cumulus cells surrounding the oocyte. Atretic follicles were defined as those follicles with more than 5% of pyknotic cells in the largest cross-section, showing oocyte shrinkage and occasional breakdown of germinal vesicle. Also included in this group were follicles showing lack of the oocyte or deformation. Quantification of zona pellucida remnants (ZPRs) previously allowed atretic

follicles to be distinguished without the complication of estimating the degree of granulosa cell apoptosis.

Immunohistochemistry

5 μm thick sections of paraffin-embedded ovarian tissues were deparaffinized, treated with citrate buffer (pH 6.0, 0.01 M) in a microwave for 3 minutes, and then treated with 0.3% hydrogen peroxide in methyl alcohol for 20 minutes to block endogenous peroxidase activity. After three washes in Tris-buffered saline (TBS; (pH 7.4), the sections were treated with 10% normal goat serum (Vector ABC Elite Kit; Vector Laboratories) and then incubated for 12 hour at 4°C temperature with primary antibody: rabbit polyclonal anti-LHR antibody (dilution 1:200; BIOSS Inc.). In the negative control, primary antibody was omitted. After three washes in TBS, the ovarian sections were incubated with the appropriate secondary antibody (dilution 1:200; Vector Laboratories) for 2 hours, washed three times in TBS, and then incubated for 45 minutes with avidin-biotin peroxidase complex (Vector ABC Elite kit), and prepared according to the manufacturer's instructions. The peroxidase was reacted using a diaminobenzidine substrate kit (Vector Laboratories). The sections were counterstained with hematoxylin, dehydrated, and cleared with xylene before finally being mounted.

Western blotting

Tissue samples were dissected, minced, homogenized (20 strokes in a homogenizer), and lysed in a buffer containing 40 mM Tris-HCl (pH 7.4) and supplemented with protease inhibitors leupeptin (10 μg/ml), phenylmethanesulfonyl fluoride (1 mM), and aprotinin (20 μg/ml). Equal amounts of protein (40 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, the resolved proteins were immunoblotted onto nitrocellulose membranes (Whatman GmbH, Dassel, Germany). The remaining binding sites on the membranes were blocked through incubation with 5% non-fat milk in TBS for 2 hour and then the membranes were incubated for 12 hours with rabbit polyclonal anti-LHR antibody (dilution 1:750; BIOSS Inc.). The membranes were washed three times in TBS containing 0.1% Tween-20 and then incubated with horseradish peroxidase conjugated anti-rabbit IgG (dilution 1:2000; Vector Laboratories) for 2 hour. Immunoreactive bands were developed using an ECL kit (Amersham) according to the manufacturer's instructions. To adjust for the amount of protein loaded and purposes of normalization, the membranes were stripped and reprobed using anti-beta-actin antibody (dilution 1:2000; Sigma- Aldrich) (17,23). The optical density of each band was measured with a laser scanner. Image J was used to analyze the intensity of the western blot bands and relative expression of LHR was calculated after normalization to the beta-actin band from each sample.

Statistical analyses

The mean ± standard error of the mean (± SEM) are used to induce data. One-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls post hoc test for multiple comparisons are used to analyze data. In our experiments, a p-value under 0.05 was considered significant.

Results

Analysis of open-field test

In the open-field test, we also took measure the anxiety level of chronic unpredictable stressed mice by checking their time of stay in the center area of open-field apparatus. The open-field apparatus is made up of a square-shaped plexiglass box with a line showing the center area. Each animal was positioned in the box for 30 minutes. The two factors were measured: the overall activity in the apparatus and the amount of time and distance traveled in the center area of the open-field. Mice instinctively feel safer near a wall rather than being exposed out in the open-field. In this evaluation, stressed mice spent significantly less time in the center area (Fig 1A), moved significantly less distances in the center area (Fig 1B) and had significantly less entries into the center area (Fig 1C) compared to controls. No significant correlations were measured in total moved distance between stressed mice and control mice (Fig 1D).

Analysis of marble-burying test

In the marble-burying test, which is used to checking anxiety-like behaviors (36), chronic unpredictable stressed mice significantly increased marble-burying behavior compared with control mice (Fig 2).

Body weight gain of control and stressed mice

Chronic unpredictable stress also effects on body weight gain. Stressed mice had decreased body weight gain during stress experiment compared to control mice (Fig 3A). After stress experiment for 30 days, body weight gain of stressed mice decreased markedly compared with that of the control mice (Fig 3B).

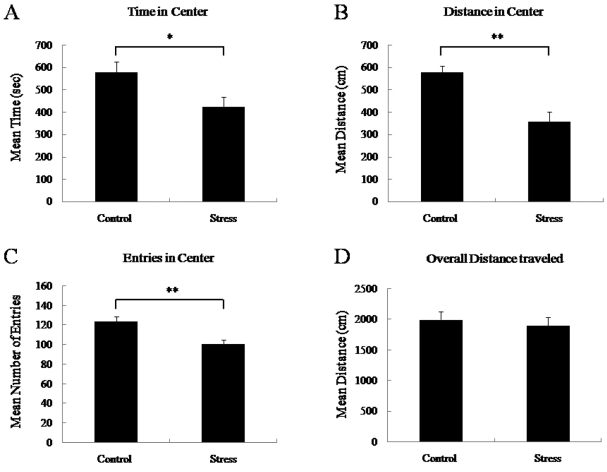

Fig 1. The effect of chronic unpredictable stress on the behavior of mice in open-field test. (A) Stressed mice spent significantly less time in the center area of the open-field compared to control mice. (B) Stressed mice moved longer distances in the center of the open-field compared to control mice. (C) Stressed mice had more entries into the center area of the open-field compared to control mice. (D) No significant statistical correlations were observed in total moved distance between stressed mice and control mice (mean ± SEM, n = 5 mice/group, *p < 0.05, **p < 0.01).

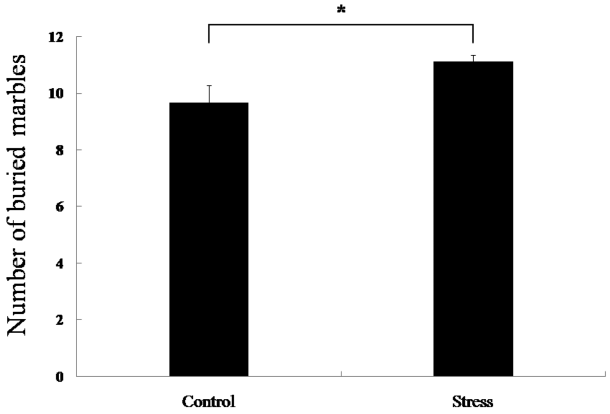

Fig 2. The effect of chronic unpredictable stress on the behavior of mice in marble-burying test. Marble-burying behavior of control group and stressed group in mice was examined twice per each group. Chronic unpredictable stress significantly increases marble-burying behavior (mean ± SEM, n = 6 mice/group, *p < 0.05).

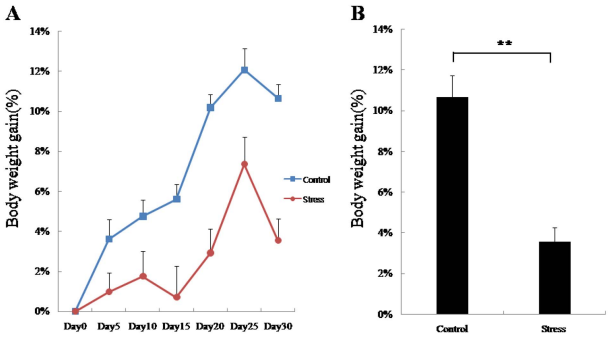

Fig 3. The effect of chronic unpredictable stress on the body weight gain. (A) Stressed mice had decreased body weight gain compared with controls for 30 days. (B) After 30 days, stressed group gained significantly less body weight compared with control (mean ± SEM, n = 7 mice/group, **p < 0.01).

Fertility test of control and stressed mice

Chronic unpredictable stress impacts average of litter size. Stressed mice had decreased average of litter size compared to controls (Fig 4B). No significant statistical correlations were observed in body weight of offspring between stressed mice and control mice (Fig 4A).

Ovarian follicular morphology

Chronic unpredictable stress impacts on the ovarian follicular development in mice. Compared with control mice, the rate of primary, secondary and early antral follicles in stressed mice significantly decreased, whereas that of atretic follicles significantly increased. There was no statistically significant difference in the rate of preovulatory follicles and corpus luteum (Fig 5). Atretic follicles were observed in the ovaries of chronic unpredictable stress induced mice. In atretic follicles, pyknotic granulosa cells (Fig 6D arrows and Fig 6E arrows) and shrinking oocytes (Fig 6D arrowhead and Fig 6E arrowhead) were observed, whereas in the well-developed control ovaries, intact granulosa cells and oocytes (Fig 6A arrowhead, Fig 6B arrowhead and Fig 6C arrowhead) were

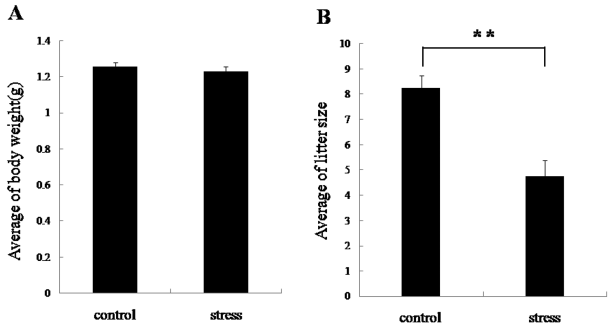

Fig 4. The effect of chronic unpredictable stress on the average body weight of offspring and average of litter size. (A) The average body weight of offspring in two groups, control dams and stressed dams, on postnatal day 1 (PND1). There were no difference in dam's body weight between control group and stressed group. (B) The average of litter size born to control group or stress group dams. Stressed group significantly decreased number of dams compared with control group (mean ± SEM, n = 4 mice/group, **p < 0.01).

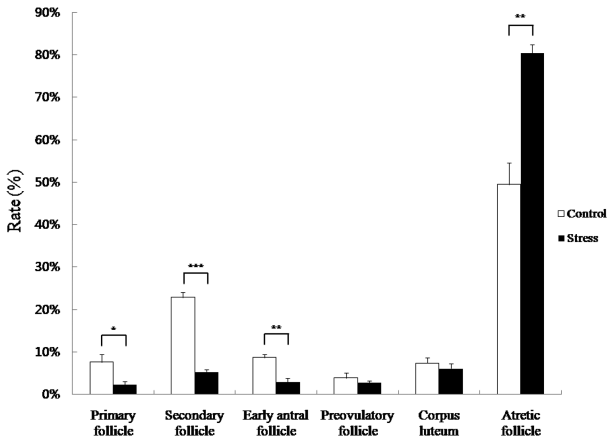

Fig 5. The effect of chronic unpredictable stress on the follicular development in mice. Compared with control mice, stress mice significant decrease in the rate of primary, secondary and early antral follicles. Although there was no significant statistical difference in the rate of preovulatory follicles and corpus luteum, there was still a no significant statistical increase in the rate of atretic follicles in stressed mice compared to control mice (mean ± SEM, n = 4 mice/group, *P < 0.05, **P < 0.01, ***P < 0.001).

observed. There were more ZPRs (Fig 6F arrows) in stressed mice, which represented end-stage atretic follicles.

Immunohistochemical analysis of LHR expression in the ovaries of control and stressed mice

In the ovaries of control mice, intensity of LHR immunoreactivity was weak in the granulosa cells (Fig 7A arrows) and weak or negative in the theca interna cells (Fig 7A arrowhead) of secondary (Fig 7A) follicles, but as follicles grow it was intense in the granulosa cells (Fig 7B and 7C arrows) and weak or moderate in theca interna cells (Fig 7B and 7C arrowheads) of early antral (Fig 7B) and preovulatory (Fig 7C) follicles. In contrast with the result on the control mice, intensity of immunoreactivity was weak in the

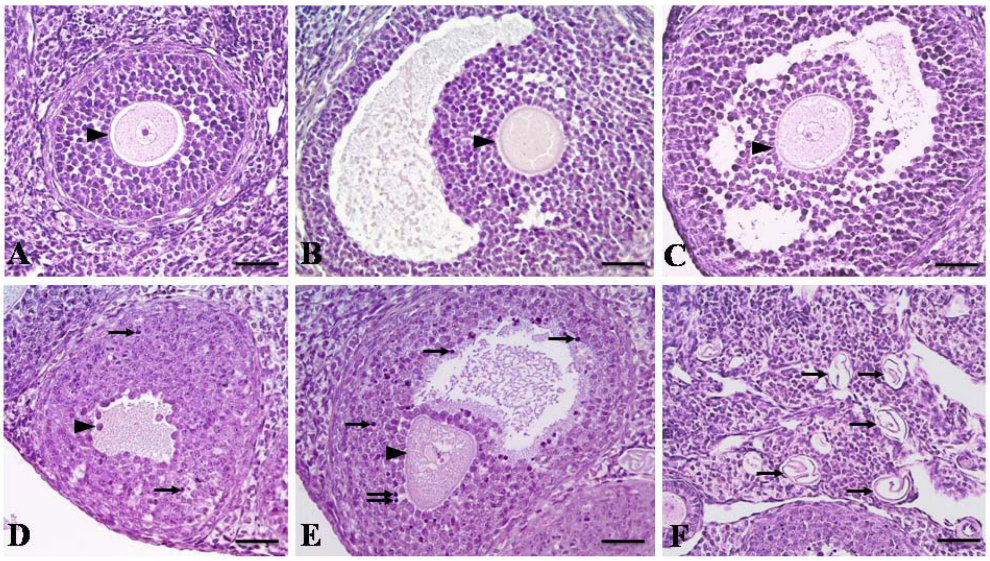

Fig 6. The effect of chronic unpredictable stress on the follicular morphology in mouse ovaries. Representative images of H&E staining of follicles in control and stressed mice. Control mice have well-developed secondary (A), early antral (B) and preovulatory (C) follicles. Intact granulosa cells and oocytes (A, B and C arrowheads) were observed in follicles of control mice. Compared with controls, stressed mice have atretic secondary (D), atretic antral (E) follicles and ZPRs (F). Pyknotic granulosa cells (D and E arrows) and shrinking oocytes (D and E arrowheads) were observed in atretic follicles of stressed mice. Zona pellucida remnants (ZPRS) represent end-stage atretic follicles (F arrows). Scale bars = 5 μm.

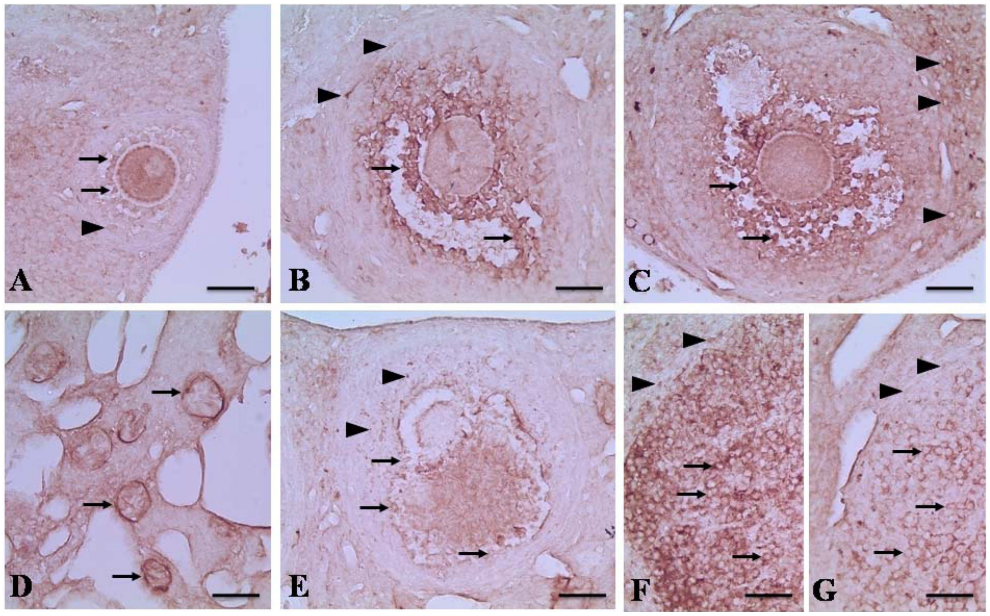

Fig 7. The effect of chronic unpredictable stress on the ovarian LHR detected by immunohistochemistry. The intensivity of the luteinizing hormone receptor (LHR) immunoreactivity in the control mice was weak in the granulosa cells (A arrows) and weak or negative in theca interna cells (A arrowhead) of secondary (A) follicles, and intense in the granulosa cells (B and C arrows) and weak or moderate in theca interna cells (B and C arrowheads) of early antral (B) and preovulatory (C) follicles. The intensity of the LHR immunoreactivity in the stressed mice was weak in the granulosa cells (E arrows) and weak or negative in the theca interna cells (E arrowheads) of atretic antral (E) follicles, and intense in the zona pellucida remnants (ZPRs) (D arrows). The intensity of expression of the LHR immunoreactivity was intense in the granulosa luteal cells (F arrows) and moderate in the theca luteal cells (F arrowheads) of control mice, whereas weak in the granulosa luteal cells (G arrows) and weak or negative in the theca luteal cells (G arrowheads) of corpus luteum (G) in the stressed mice. Scale bars = 5 μm.

granulosa cells (Fig 7E arrows) and weak or negative in the theca interna cells (Fig 7E arrowheads) of atretic antral follicle (Fig 7E) of stressed mice, but intense in the ZPRs (Fig 7D arrows) in the ovaries of stressed mice. In the corpus lutea, expression intensity of LHR was intense in granulosa luteal cells (Fig 7F arrows) and moderate in the theca luteal cells (Fig 7F arrowheads) of the control mice, whereas it was weak or moderate in the granulosa luteal cells (Fig 7G arrows)

Table 1. Localization and intensity of LHR in granulosa cells, theca interna cells and luteal cells of control group and stressed group

Region	Tissue or cell type	LH receptor
Intact primary follicle	Granulosa cell	–
	Theca interna cell	–
Intact secondary follicle	Granulosa cell	+
	Theca interna cell	– or +
Intact early antral follicle	Granulosa cell	+++
	Theca interna cell	+ or ++
Intact preovulatory follicle	Granulosa cell	+++
	Theca interna cell	+ or ++
Corpus luteum of control mice	Granulosa luteal cell	+++
	Theca luteal cell	++
Atretic primary follicle	Granulosa cell	–
	Theca interna cell	–
Atretic secondary follicle	Granulosa cell	–
	Theca interna cell	–
Atretic early antral follicle	Granulosa cell	+
	Theca interna cell	– or +
Atretic preovulatory follicle	Granulosa cell	++
	Theca interna cell	– or +
Corpus luteum of stress mice	Granulosa luteal cell	+ or ++
	Theca luteal cell	– or +
End stage of atretic follicle	ZPR	+++

Stained sections were scored for the density of positive cells, per field.
–, negative; +, weak; ++, moderate; +++, intense.

and weak or negative in the theca luteal cells (Fig 7G arrowheads) of stressed mice.

Table 1 summarizes the immunohistochemical analysis of LHR in granulosa cell, theca interna cell and luteal cells of control mice and stressed mice.

Western blot analysis of LHR expression in the ovaries of control and stressed mice

The western blot analysis revealed significantly decrease in LHR expression in the stressed mice ovaries compared with the control (Fig 8). Western blot analysis mostly confirmed the results of the immunohistochemical analysis.

Discussion

In this study, psychosocial stress was given to mice by pushing unpredictable stress, mimicking a series of actual life events. The initial activity of laboratory animal placed in the open-field has considered as an indicator of its psychological and emotional state (47). Stressed animals stayed less in center of the open-field, indicating anxiety-like activity. Decreased activity and exploratory action speaks for a loss of interest in novel, stimulating events, suggesting a decrease in motivation (32). The open-field test of the mice in our present study indicates that the mice were feeling emotionally and psychologically anxious after chronic unpredictable stress (Fig 1). The marble-burying test is used to test anxiety-related behaviors (36). Marble-burying is normally regarded as a model of anxiety disorders characterized by compulsive and repetitive behaviors including an obsessive-compulsive disorder (38). In our present study, chronic unpredictable stressed mice significantly increased marble-burying behavior compared with control mice (Fig 2). The result from open-field test and the marble-burying test suggested that the stress model was established successfully.

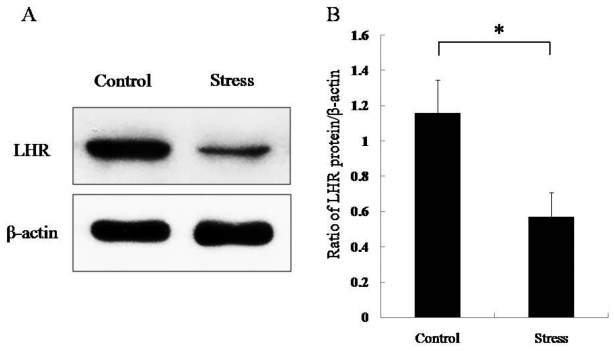

Fig 8. The effect of chronic unpredictable stress on the ovarian LHR detected by western blot analysis. (A) Representative images of the Western blot analysis for LHR and beta-actin. (B) Western blot analysis revealed significantly decrease in LHR expression in the stressed mice ovaries compared with the control (mean ± SEM, n = 4 mice/group, *$p < 0.05$).

Chronic unpredictable stress also effects on body weight gain. Stressed mice had attenuated body weight gain during stress experiment compared to controls. The results indicate that decreased body weight gain effects that have been formerly reported for various chronic stress models (9,35,39); therefore, it appears that the chronic unpredictable stress model used in these experiments was an effective stress experiment (Fig 3).

Levels of active glucocorticoids caused by stress are impeded during ovarian follicular growth and maturation, whereas levels are increased during the ovulation process induced by the LH surge. Furthermore, increase in levels of glucocorticoids caused by stress negatively influences fertility in women. Increased levels of glucocorticoids damaged not only ovarian function but also uterine (41). In the present study, the average of litter size decreased in chronic unpredictable stress model (Fig 4). The results indicated that chronic unpredictable stress not only suppresses ovarian follicular growth and development but also decrease fertility rate.

Previous research showed that chronic intermittent cold stress reduces the number of secondary and antral follicles, whereas the number of atretic follicles is not affected in the ovaries of rats (13). In chronic unpredictable stress model study by Wu *et al*, stress not only inhibits secondary and antral follicular development, but also induces atresia of growing follicles. In our present study, not only chronic unpredictable stress inhibits primary, secondary and early antral follicular growth, but it also increases atresia of growing follicles, whereas there was no statistical significant difference in the rate of preovulatory follicles and corpus luteum (Fig 5).

In atretic follicle, granulosa layer became loose and the pyknotic cells appeared. Since then, granulosa cells were

eliminated massively and floated in the antrum. Furthermore, the oocyte shrank to a certain extent, and the cumulus granulosa cells receded from the zona pellucida (ZP). The oocyte completely degenerated and ZPRs are observed. The ZP is a glycoprotein membrane surrounding the plasma membrane of an oocyte. ZP first appears in primary follicles. ZP is one of the last components in degenerated atretic follicle that persists after the death of the granulosa cells and oocyte (8,21,27). The number of ZPRs was provided an indicator of those growing follicles that had developed a ZP, but had subsequently experienced atresia. It is one of the classifications that can be used to determine those follicles. Quantification of ZPRs can be distinguished without the complication of estimating the degree of granulosa cell apoptosis (27). In present study, we found that the rate of atretic follicles including ZPRs in stressed mice significantly increased compared with control mice.

It has been reported that there is the expression of LHR on the theca interna layer in small human follicle (34). Also there is the expression of LHR on the theca interna cells in the preantral, antral and preovulatory follicles detected by indirect immunofluorescence (37). The immunoreactive LHR detection on theca interna cells in preantral and small antral follicles indicates the participation of LH in the synthesis of androgen in these follicles and sustains the participation of endocrine and reproductive systems in early follicular growth (37). In our present study it is shown that the intensity of LHR immunoreactivity was weak or moderate in the theca cells of early antral follicle and preovulatory follicle, whereas it was weak or negative in the theca cells in the preantral follicles. We supposed that the intensity of LHR expression may be discrepancy between in vivo and in vitro experiment.

The intensity of LHR immunoreactivity was weak or negative in the theca cells of the atretic antral follicles. This result suggests that chronic unpredictable stress can give negative effects in the early follicular development.

As follicles grew in the control ovaries, the intensity of LHR immunoreactivity in the granulosa cells has grown intensier in our present study. This result is compatible with the previous study (37). The major functions of granulosa cells are sex steroids production and multiple growth factors thought to interplay with the oocyte throughout its development. Granulosa cells of preovulatory follicle are considered able to react with the signal of mid-cycle LH surge and start to produce corpus lutea. These phenomenons indicate that granulosa cells have acquired a sufficient LHR on their cell surface (37). In our present study, it is shown that the expression of LHR on granulosa cells increased before ovulation. Whereas it was weak in the granulosa cells of atretic antral follicles, which can suggest that stress can effect on follicular development and ovulation.

Since LH induces production of progesterone by luteinizing granulosa cells (16), endogenous LH may induce production of progesterone by luteinizing granulosa cells via LHR during the formation of corpus luteum. In our present study, it is shown that intensity of LHR in the granulosa luteal cells was weaker in the stressed mice than in the control mice. This suggests that stress can give effects on the production of granulosa luteal cell progesterone and pregnancy maintenance.

Synthesizing these results, there is a significant decrease of intensity of LHR expression in theca cells, granulosa cells and luteal cells in stressed mice by chronic unpredictable stresses. Also there was a significant decrease in litter size in stressed mice. These findings suggest that chronic unpredictable stresses decreased the LHR expression of ovaries which is related to follicular development, ovulation, and pregnancy maintenance in stressed mice.

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실험적 마우스 모델에서 예측 불가능한 스트레스가 황체형성호르몬 수용체의 발현과 생식기능에 미치는 영향에 관한 연구

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요 약 : 본 연구의 목적은 예측 불가능한 만성적인 스트레스가 생식기능과 황체형성호르몬 수용체의 발현에 미치는 영향을 알아보는 것이다. 9 주령 암컷 C57BL/6 마우스를 무작위로 선택하여 대조군과 스트레스군의 두 집단으로 분류하였다. 스트레스군은 35일 동안 하루에 두 번씩 12가지의 서로 다른 스트레스를 무작위로 선택하여 스트레스를 주었다. 대조군에 비하여 스트레스군에서 불안과 관련된 행동들이 유의성 있게 증가하였으며($P < 0.05$), 스트레스를 받는 동안의 증체율 또한 유의성 있게 감소하였다($P < 0.01$). 그리고 평균 산자수도 대조군에 비하여 스트레스 군에서 유의하게 감소함을 관찰 하였다($P < 0.01$). 조직학적인 검사에서 일차, 이차 및 초기 성숙 난포의 비율이 대조군에 비해 스트레스 군에서 유의하게 감소한 반면($P < 0.05$) 폐쇄 난포의 비율은 유의하게 증가하였다($P < 0.01$). 면역조직화학적 검사를 통해 과립막세포와 황체세포의 황체형성호르몬 수용체 발현을 관찰한 결과, 대조군에 비해 스트레스군에서 그 발현이 감소하였고, 웨스턴 블롯을 통해 난소 내 황체형성호르몬 수용체의 단백질 양을 측정한 결과 또한 대조군에 비하여 스트레스군에서 유의하게 감소하였다($P < 0.05$). 본 연구를 통해 난소의 황체형성호르몬 수용체는 예측 불가능한 스트레스에 의해 영향을 받으며, 변화된 황체형성호르몬 수용체가 난자의 난포 발육 불량과 생식기능의 이상에 영향을 미친다는 것을 실험적으로 증명하였다.

주요어 : 만성 스트레스, 난포 발육, 황체형성호르몬 수용체, 스트레스 관련 행동