**Gender and age differences in the suppressive effect of a 50 Hz electric field on the immobilization-induced increase of plasma glucocorticoid in mice**

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**ABSTRACT**

We developed an experimental system to characterize the suppressive effect of extremely-low-frequency (ELF) electric fields (EFs) on the stress response. We assessed differences in the EF effects by age and gender. Control, EF-alone, immobilization-alone, and co-treated groups were subjected to an EF (50 Hz, 10 kV/m). Co-treated mice were exposed to the EF for 60 min, with immobilization during the latter half. Our results indicate that the suppressive effects of ELF EFs on the stress response in immobilized mice occur regardless of gender or age. Because stress plays an important role in the onset and progression of various diseases, these findings may have broad implications for understanding the efficacy of EFs in animal, and perhaps human, health.

Keywords: stress; endocrine response; extremely low frequency; electrical stimulation; ovariectomy

## INTRODUCTION

The widespread use of electricity in domestic and industrial settings highlights the importance of investigating the biological effects of extremely-low-frequency (ELF) electric fields (EFs), particularly at 50 and 60 Hz power-line frequencies [WHO, 2008]. However, ELF EFs have been applied in clinical treatment and health maintenance, including in medical facilities and homes, for at least half a century in Japan [Harakawa et al., 2014; Ito, 2000; Mitani et al., 2015; Shinba et al., 2012]. Some studies have shown that EFs may have therapeutic effects, for example, to treat bone diseases, stimulate bone growth, or heal wounds [Aaron et al., 2006]. In 1979, the United States Food and Drug Administration approved devices that apply an EF to the skin surface, resulting in induced electric currents that mediate desired therapeutic effects [FDA, 2006]. Electric currents induced in the body and the perception of EFs at the skin surface can trigger cellular and humoral responses in certain organisms [Kato et al., 1989; Romo et al., 1998, 2000, 2002; Weigel et al., 1987; Weigel and Lundstrom, 1987]. However, their biological effect and influence are not yet clearly understood. Owing to the relatively facile shielding from EF, the effects of exposure to ELF magnetic fields may be particularly important in humans. However, better understanding of the biological effects of EFs is needed in order to use EFs to maintain and improve health.

We previously established an experimental system to evaluate the efficacy of ELF EFs of at least at 50 Hz in reducing the stress response induced by immobilization in rodents

[Harakawa et al., 2004, 2005, 2017; Hori et al., 2015, 2017]. We found that the effects of EFs on plasma glucocorticoid (GC) were amplified when the body surface area exposed to EFs was relatively large, whereas the effects were diminished when the body surface area exposed to the EF was relatively small [Hori et al., 2018].

Recently, we found that in BALB/c male mice the immobilization-induced increase of GC levels was reduced by exposure to an EF of 50 Hz when the voltage was applied via the upper electrode of a parallel plate electrode system. This effect was dependent on both intensity (kV/m) and exposure time [Hori et al., 2015]. Furthermore, the effect was pronounced at 20 min after the initiation of immobilization [Harakawa et al., 2017], and this effect might depend on the intensity distribution of the EF on the body surface, even when the EF strength remains unchanged [Hori et al., 2017]. Furthermore, the suppressive effect is influenced by environmental illuminance [Hori et al., 2018].

Here, we examine whether the suppressive effect of an EF on the immobilization-induced increase in GC level differs between the sexes, or between mice varying in age.

## MATERIALS AND METHODS

Male mice (8, 13, 23, and 53 weeks old), intact female mice (8 weeks old), and ovariectomized female BALB/c mice (8 weeks old) were purchased from Charles River Japan (Kanagawa, Japan) and maintained in a pathogen-free environment at 24 ± 1 °C and 50 ± 10% humidity with daily artificial illumination (12:12-h light/dark cycle with lights on

from 07:00–19:00 h). The animals had free access to standard laboratory chow (CE-2; CLEA, Tokyo, Japan) and water, except during the period of immobilization and EF exposure.

Animal experiments were carried out in accordance with the Guiding Principles for the Care and Use of Research Animals of Obihiro University of Agriculture and Veterinary Medicine, Japan. The protocol was approved by the Committee on the Ethics of Animal Experiments of Obihiro University of Agriculture and Veterinary Medicine (permit number 26–119 and 27-132).

The EF exposure system (Fig. 1) consisted of three parts: a high-voltage transformer unit (maximum output voltage, 30 kV; Hakuju, Tokyo, Japan), a constant-voltage unit (CVFT1-200H; Tokyo Seiden, Tokyo, Japan) to avoid unexpected interference from electrical noise originating from the commercial power supply, and a parallel plate electrode system [Harakawa et al., 2017; Hori et al., 2017, 2018]. The parallel plate electrode system comprised a cylindrical plastic cage (diameter, 200 mm; height, 100 mm) and two stainless steel electrodes (1,000 × 600 mm) that were placed over and under the cylindrical exposure cage (Fig. 1). The cylindrical cage had slits (length, 100 mm; width, 5 mm) all around at intervals of 5 mm (Fig. 1) to prevent smudges (from feces or saliva) from disturbing the formation of a stable EF. A separate exposure cage and immobilization tube were used for each animal. The cages and tubes were reused after they were washed with a neutral detergent and completely dried. Using a digital thermometer placed on the lower electrode, the temperature was measured before EF exposure and 10, 20, 30, 40, 50, and 60 min after

EF exposure. The temperature inside the cage was 25 ± 3 °C during the period of EF or sham exposure. The temperature within the cylindrical cage did not change during either period. The humidity was maintained between 45 and 55%.

Mice in the EF treated groups were exposed to an EF of 10 kV/m for 60 min. To generate the EF of 10 kV/m in the cage, 50 Hz, 1 kV was applied to the upper electrode, whereas the counterpart electrode was grounded. To measure the field intensity and verify the system's operation, an optical fiber voltmeter, which measures EF intensity by the Pockels effect, and an electro-optic voltage sensor attached with a two-cored $Bi_{12}SiO_{20}$ fiber (FOVM 03; Sumitomo Electric, Osaka, Japan) and a digital multimeter (Fluke 87; Fluke, Everett, WA) were used. The EF intensity was measured at 273 arbitrary points (21 × 13) on each cage floor. The 10 kV/m EF intensity applied to the cage had a margin of error of ± 4% (outside the exposure cage) and ± 0.1% (inside).

To measure the magnetic field intensity in the area where each mouse was exposed to the EF, a portable alternating current magnetic field meter (TMM-1, Electric Power Engineering Systems, Kanagawa, Japan) was used. The magnetic field intensity was approximately 0.12 ± 0.04 mG when a 50-Hz, 10 kV/m EF was generated in the space.

To examine the effect of EF exposure with or without immobilization stress on plasma GC level, 10-week-old male, female, and ovariectomized female mice were divided into four groups (n = 6 each): a control group [Stress(–)/EF(–)], EF-alone group [Stress(–)/EF(+)],

immobilization-alone group [Stress(+)/EF(−)], and co-treated group [Stress(+)/EF(+)] (Fig. 3).

All mice spent 60 min in the EF exposure cage. Mice in the control group were housed in the EF cage for 60 min without exposure to an EF (i.e., 0 kV/m). Mice in the EF-treated groups were all exposed to an EF for 60 min.

Stress was applied by immobilizing each mouse separately within a 50-mL centrifuge (polypropylene) tube that was placed on the lower electrode (Fig. 1B) [Hori et al., 2015, 2018]. Immobilization stress was applied during the latter half (30 min) of the 60-min EF test period. The EF intensity in the device after immobilization was kept at over 95% of the initial intensity.

To examine age impact on the effect of the EF on stress-induced plasma GC levels, 15- (young adult), 25- (adult), and 55- (older adult) week-old mice (mass: 26.4–30.0 g, 29.7–34.2 g, and 32.5–37.5 g, respectively) were randomized into four treatment groups for each age group (n = 6 per group): a control group [Stress(–)/EF(–)], a EF-alone group [Stress(–)/EF(+)], immobilization-alone group [Stress(+)/EF(−)], and co-treated group [Stress(+)/EF(+)] (Fig. 2).

All mice spent 60 min in the EF exposure cage. Stress was applied by immobilizing each mouse separately within the devices, which were adjusted for each mouse depending on its age. As can be seen in Fig. 3B, each mouse was placed on the lower electrode. The

immobilization device consisted of four parts: the main body, a polypropylene spacer to limit back-and-forth movement, a Teflon stopper (SK. Tokyo, Japan), and a polypropylene hoop (Fig. 3A). The main body of the immobilization device included slits (4 mm) whose respective diameter and length were 29 mm and 130 mm for the 15-week-old mice, 31 mm and 132 mm for the 25-week-old mice, and 33 mm and 138 mm for the 55-week-old mice, with a thickness of 1 mm. The diameter of the spacer was 29 mm, 31 mm, and 33 mm, respectively, for the 15-, 25-, and 55-week-old mice with a length of 35 mm. Immobilization stress was applied during the second half (30 min) of the 60-min EF test period (Fig. 2). The EF intensity of the device after immobilization decreased by approximately 4%. Because a spatial EF within 2 cm of the Teflon stopper can influence the results, the mouse and the Teflon stopper were kept apart by a distance exceeding 2 cm.

Immediately after EF treatment (between 10:00 and 12:30), we collected 800 μL of blood from the heart atrium of each mouse under 3% isoflurane anesthesia (Mylan, Tokyo, Japan) using a syringe with a 25-gauge needle. Immediately afterward, 10 μL of the blood sample was used to analyze blood properties with a Celltak system (Nihon Kohden, Tokyo, Japan). The remainder of the sample was centrifuged at 1,500 × g for 10 min at 4 °C, and the plasma was collected and stored at −80 °C until use.

To derivatize GC, the plasma (200 μL) was mixed with 900 μL of isooctane (2,2,4-trimethylpentane; Wako, Osaka, Japan) followed by vortexing and centrifugation at 380 × g for 5 min at 25 ºC. The upper layer was discarded, and 900 μL of chloroform (Wako) was

added to the lower layer. The sample was vortexed and centrifuged at 380 × g for 5 min at 25 ºC. The upper and white membranous layers were removed, and the lower layer was retained for analysis; 800 µL were transferred to a new tube and mixed with 320 µL of a solution containing 65% concentrated sulfuric acid and 35% ethanol (both from Wako), followed by vortexing. The solution was incubated in the dark for 3.5 h, and the fluorescence intensity of the sample was measured at 519 nm with excitation at 475 nm using a spectrofluorophotometer (RF-5300PC; Shimadzu, Kyoto, Japan). Sulfuric acid-induced fluorescence of glucocorticoid was used to measure the total glucocorticoid [Silber et al., 1958; Zenker and Bernstein, 1958].

To assess the association between treatment type and differences in plasma level of glucocorticoid depending on gender or age, two-way ANOVA was used, followed by Bonferroni's multiple comparison test. Correlation and regression analysis were performed using Pearson's correlation analysis and slope regression analysis. Significance was defined as $P < 0.05$. All statistical analyses were conducted using Prism Version 8 (GraphPad Software, La Jolla, CA).

**RESULTS**

There was no difference in plasma GC level among male mice in the EF-alone (50 Hz, 10 kV/m for 60 min) and control groups (Fig. 4A). However, plasma GC level was higher in mice that were only immobilized [Stress(+)/EF(–)] compared to controls [Stress(–)/EF(–), $P < 0.0001$; Fig. 4A]. The GC level in the co-treated group [Stress(+)/EF(+)] was higher than

that in control mice but was lower than that in the immobilization-alone group ($P < 0.05$; Fig. 4A). Similar trends were observed in female mice; however, females had higher plasma GC levels than males overall ($P < 0.0001$ by two-way ANOVA; Fig. 4A).

There was no difference in the white blood cell (WBC) count between male and female mice in each group (Fig. 4B). However, red blood cell (RBC) count, hemoglobin (HGB) level, and hematocrit (HCT) values were higher in females than in males ($P < 0.0001$ by two-way ANOVA; Fig. 4C-E). The RBC count, HGB level, and HCT were lower in both male and female control mice than in the corresponding treatment groups. There were no differences in any of the measured blood properties among the immobilization-alone and co-treated groups, except in terms of HGB level ($P < 0.05$ by Bonferroni's multiple comparison test; Fig. 4).

In all age groups, no correlation was observed between the body mass and GC levels (Fig. 4F). Significant differences in plasma GC were observed among ages ($P < 0.05$) and treatments ($P < 0.0001$). In the 15-week-old mice, there were no significant differences in plasma GC between mice in the EF-alone [Stress(–)/EF(+)] group and the control [Stress(–)/EF(–)] group. In the 25- and 55-week-old mice, however, the plasma GC levels of mice in the Stress(–)/EF(+) group were higher than those of the mice in the control group ($P < 0.0005$ and $P < 0.0005$, respectively). The GC levels in the control group exhibited a decreasing trend depending on the age ($r = -0.999$, $P < 0.05$ by correlation analysis, but not significant by linear regression analysis). In other treatment groups, no correlation with age was observed (Fig. 4F).

In all age groups, GC levels were significantly higher in the groups that included immobilized mice than in the control group ($P < 0.0001$ by Bonferroni's test), while they were lower in the co-treatment [Stress(+)/EF(+)] group than in the immobilization-alone [Stress(+)/EF(–)] group ($P < 0.0001$ at 15 weeks old, $P < 0.005$ at 25 weeks old, $P < 0.05$ at 55 weeks old) (Fig. 4F).

In terms of blood properties, WBC and platelet counts did not show any correlation with age, and no differences were observed among the different groups (Fig. 4G). RBC counts did not show any correlation with age, and values were higher in the immobilized mice than in the control mice ($P < 0.0001$ for all age groups). HCT did not show any correlation with age, and values were higher in the immobilized mice than in the control mice ($P < 0.05$ at 15 weeks old, $P < 0.01$ at 25 weeks old, $P < 0.0005$ at 55 weeks old). There were no differences in either RBC counts or HCT between the immobilized mice and the co-treated mice. HGB levels did not show any correlation with age except for the co-treatment group ($P < 0.05$ by correlation analysis, but not significant by linear regression analysis), and values were higher in the immobilized mice than in the control mice ($P < 0.05$ at 15 weeks old, $P < 0.005$ at 25 weeks old, $P < 0.01$ at 55 weeks old). There was no difference in HGB levels between the immobilized and co-treated mice.

## DISCUSSION AND CONCLUSIONS

This study assessed whether the suppression of immobilization-induced increases in plasma GC levels by EF exposure is influenced by either gender or age differences.

In all experiments, we selected an EF intensity of 10 kV/m based on a previous study where EF intensities ranging from 2.5–200 kV/m were assessed, and 10 kV/m showed the highest anti-stress effect of an EF at 50 Hz [Hori et al., 2015]. An additional reason for selecting an EF intensity of 10 kV/m for 60 min was that EFs with an intensity greater than 50 kV/m generated vibration and/or noise in the previous study. We noted that the effects of vibrations and/or noise are difficult to distinguish from those of the EF [Hori et al., 2015], thereby deterring the exclusion of a possible artifact at higher intensities. Therefore, this issue needs to be addressed and resolved so that higher EF intensities can be further investigated.

Our finding that plasma GC levels were higher in control females compared to control male mice is consistent with other studies [Goel and Bale, 2008; Romeo et al., 2013]. We found that EF exposure suppressed the increase in GC level induced by immobilization in male mice (Fig. 4A). Similarly, EF exposure blocked the elevation of GC level both in stressed intact female and ovariectomized female mice (Fig. 4A). These findings provide insight into sex differences in the biological effects of EF exposure, although it is limited to endocrine changes in ovariectomized mice subjected to acute stress. The data presented here are consistent with the results of our previous studies [Harakawa et al., 2004; Hori et al., 2015].

To assess the degree to which sex influences the basal plasma GC level upon EF exposure, we calculated the mean GC value in each group ([Stress(+)/EF(–)] – [Stress(–)/EF(–)])/([Stress(+)/EF(–)] – [Stress(+)/EF(+)]), which was 0.31, 0.34, and 0.29 for

males, ovariectomized females, and intact females, respectively (Fig. 4A). Although these values are similar, further studies with larger sample sizes are warranted to determine whether there is a true sex difference.

In both the gender and age tests, plasma GC levels in immobilized mice [Stress(+)/EF(–) group] were approximately two to three times higher than those of the control [Stress(–)/EF(–)] group (Fig. 4A and F), suggesting that the immobilization procedure affected the hypothalamic-pituitary-adrenal axis of the endocrine system and the sympathetic-adrenomedullary system, which manifested as stress [Kvetnansky et al., 1979; Selye, 1946].

The GC level was 0.34 ± 0.12 μg/mL in the control group and 0.43 ± 0.09 μg/mL in the EF-alone group (1.26 times higher) for the 15-week-old mice. While the hormone level under EF treatment seems to be higher than that under sham treatment, our data did not reveal a significant difference. Because there was no difference in the GC level between the EF-alone group compared to that of the control group (Fig. 4F), we conclude that an EF exposure of at least 50 Hz, 10 kV/m for 60 min did not activate the pituitary-adrenocortical axis or the sympathetic-adreno-medullary system in the 15-week-old mice in this study. Therefore, the change induced by the EF is very small in comparison to that induced by immobilization.

By contrast, in a former study, the plasma level of corticosterone (41.9 ± 22.8 ng/mL) in 6-month-old male mice increased by approximately 3.26 times following exposure to an EF of 10 kV/m for 22 h [de Bruyn and de Jager, 1994]. In the 25- and 55-week-old mice in this

study, the GC levels in the EF-alone group were significantly higher than those in the control group (Fig. 4F), indicating the possibility that EF exposure acted as a stressor as suggested by de Bruyn and de Jager (1994). However, the difference in GC levels seems to be caused by an age-dependent decrease in the control group, and an age-related decrease is well-known for a basal level of plasma GC. Thus, we have to consider the possibility that EF does not act as a stressor.

Because there was no correlation between the body mass and GC levels in the control [Stress(–)/EF(–)], immobilization-only [Stress(+)/EF(–)], and co-treatment [Stress(+)/EF(+)] groups in all age groups (data not shown), body mass did not influence the effect of EFs in this study. Exposure to an EF of 10 kV/m showed the GC-suppressive effect with high reproducibility in not only 15-week-old mice but also in 25- and 55-week-old mice (Fig. 4F). This means that the EFs used in this study should reduce the stress response induced in immobilized mice despite differences in age. This also indicates that similar effects should occur when the body mass is between 26 and 37.5 g. The mean value of plasma GC levels in [Stress(+)/EF(+)] group divided by the value of [Stress(+)/EF(–)] group was 0.73 for 15-week-old mice, 0.80 for 25-week-old mice, 0.83 for 55-week-old mice. Although further studies are needed to verify this hypothesis, it seems the suppressive effect of EF exposure decreases depending on age.

It is thought that suppression of the release of GC from the adrenal cortex or enhancement of degradation of GC in the liver could be mechanisms for decreasing the GC level by EF.

Our latest study supports the possibility that this effect can be attributed to the suppression of GC secretion [Harakawa et al., 2017]. As a mechanism for suppression of GC secretion, the release of GC from the adrenal cortex may be directly suppressed, or the upstream stress response may be suppressed. Previous studies suggested that high-intensity and chronic exposure to ELF-MF increases corticosterone secretion, along with depression and/or anxiety-like behavior, without enhancing the hypothalamic-pituitary-adrenal axis [Kitaoka et al., 2013, 2016]. However, because of the apparent anti-stress effects of ELF EFs owing to adrenocorticotropic hormone (ACTH) secretion in the pituitary gland [Harakawa et al., 2004], the hypothalamic-pituitary-adrenal axis would participate in the ELF EF-induced suppressive effect on GC levels in immobilized mice. In addition, it should be considered that EFs might have negative effects on the stress response, regardless of its function as a survival mechanism, including immunoprotection from acute stress [Dhabhar, 2009]. Thus, it should be carefully considered whether the stress response functions normally in mice exposed to ELF EFs.

Given that gender differences did not have a significant effect on the suppressive effect of the EF with regard to stress responses, it was assumed that age differences could be a factor for consideration. Thus, we consider that the experimental system, conditions, and experimental groups in the present study are representative of studies investigating the biological effects of EFs on other organisms, although the method must be developed further before extrapolation to humans. A question arises: can this treatment ever be applied

practically? Further studies are necessary to answer this question.

Although the mechanism is unknown, the RBC count, HGB level, and HCT increased by immobilization, which was associated with elevated GC level (Fig. 4C-E and H-J), We speculate that the elevation in these blood parameters can be mainly attributed to either water deprivation due to stress-induced vasoconstriction or the release of erythrocytes stored in the spleen or liver. One thing that is certain at present is that whereas EFs can cause acute stress-related changes in the endocrine system, these effects are not sufficient to impact blood properties.

To further clarify our biological findings, dosimetric studies using high-level EF exposure with rodents will be essential. Considering the current dosimetric studies reported in the literature, previous articles provide a clear description of the degree of EF and induced current in human subjects according to the World Health Organization [WHO, 2008; Tarao et al., 2012], and for studies conducted on small animals [Dawson et al., 2003; Free et al., 1981; Hart, 1992; Kaune and Phillips, 1980]. Environmental Health Criteria from the WHO [2008] provide information regarding the induced EFs (mV/m) of a grounded human body model in a vertical uniform EF of 1 kV/m at 60 Hz or 50 Hz.

One limitation of this study is that only one stress hormone (GC) was investigated. Therefore, to clarify the mechanism underlying the effect of EF exposure, we shall direct future research toward an examination of changes in the heart-rate or body temperature as measures of an anti-stress response in mice exposed to EFs, and toward an investigation into

the effects of EFs on hormones that are induced in the upstream pathway of the stress response. Furthermore, GC receptor gene transcript levels after EF, stress, or co-treatment should be investigated as a downstream biological effect of EF exposure.

In conclusion, it was demonstrated that EF suppressed immobilization-induced elevations in a stress hormone not only in male but also in intact female and ovariectomized female mice, at least with respect to the endocrine response to acute immobilization stress in mice. Because of the limitations of this study and the unknown effects of either repeated or long-term EF exposure, further studies are needed in order to test the effects of EF exposure on the endocrine system over days or weeks before determining its safety and role as acute stressor. Our findings answer fundamental questions about how ELF EFs differentially affect the two sexes. In addition, our findings confirmed that exposure to ELF EF suppresses immobilization-induced changes in the stress response despite differences in the age of mice.

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**Figure legends**

Figure 1. Electric field (EF) exposure system. A: Voltage generator and electrodes. B: EF exposure cage. C: Mouse restrained in a 50-mL centrifuge tube.

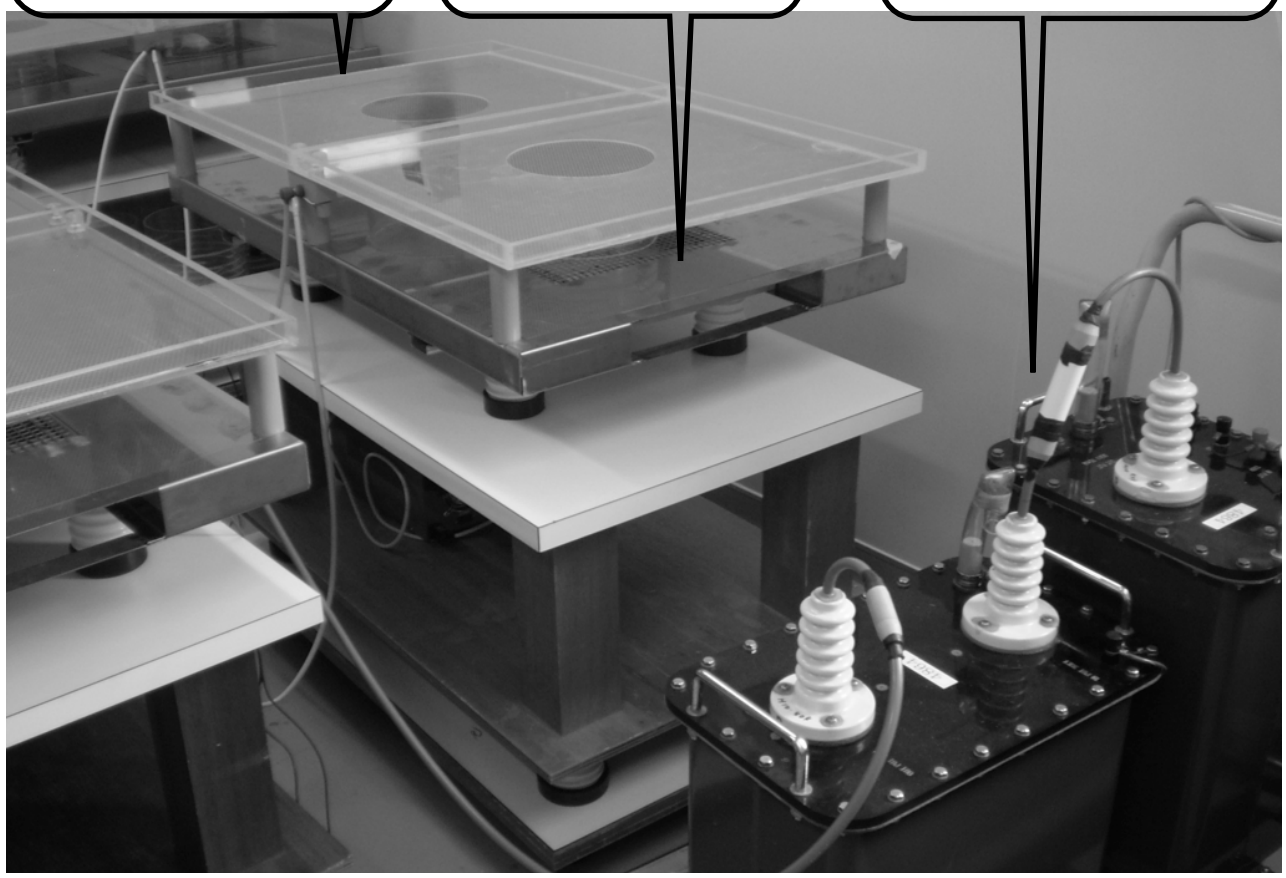
Figure 2. Experimental design for the two studies to investigate the suppression of immobilization-induced increases in plasma GC levels by EF exposure in mice. Mice were divided into the following groups (n = 6 per group): control; EF-alone; immobilization-alone; and co-treated. All mice spent 60 min in the EF exposure cage. Mice in the stress groups were immobilized for the second half (30 min) of the 60-min test period.

Figure 3. Immobilization device for age-based tests. A: Main body, spacer, hoop, and stopper. B: Immobilized mice on the lower electrode. C: Set up for 15-, 25-, and 55-week-old mice.

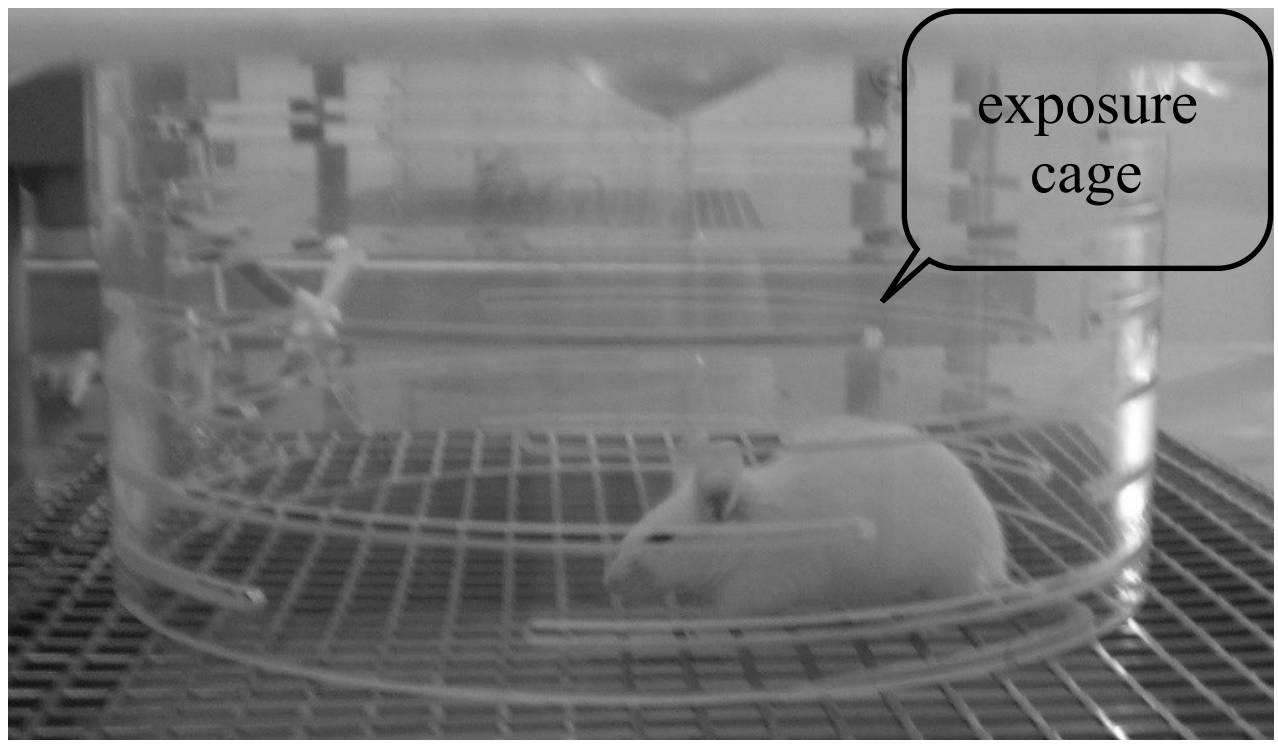
Figure 4. A: Effect of immobilization on plasma GC level and effect of EF on the immobilization-induced increase in plasma GC level in male, ovariectomized female, and intact female mice. B-E: Effect of immobilization and EF exposure on the immobilization-induced increase in plasma blood properties in male, ovariectomized female, and intact female mice. F: Effect of immobilization on plasma GC level and effect of EF on the immobilization-induced increase in plasma GC level in 15-, 25-, and 55-week-old mice.

G-J: Effect of immobilization and EF exposure on the immobilization-induced increase in plasma blood properties in 15-, 25-, and 55-week-old mice.

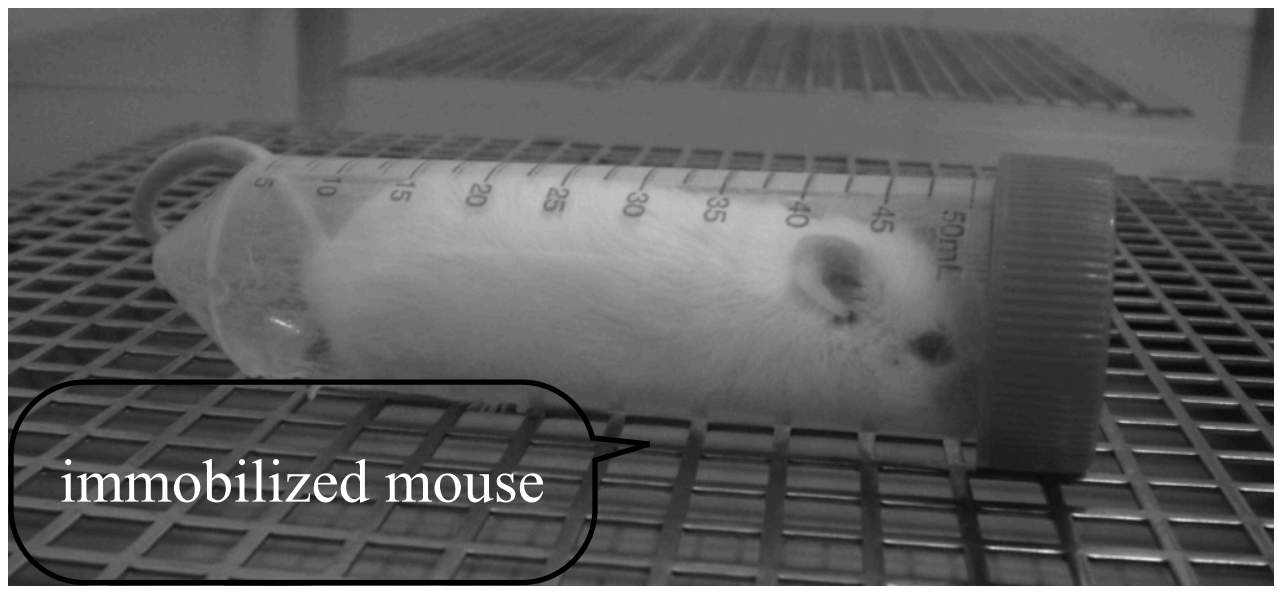
A


upper electrode
lower electrode
voltage generator

B


exposure cage

C


immobilized mouse

Receive of animal

↓

Acclimation period in SPF conditions for 2 weeks

↓

Randomization

↓

Treatment

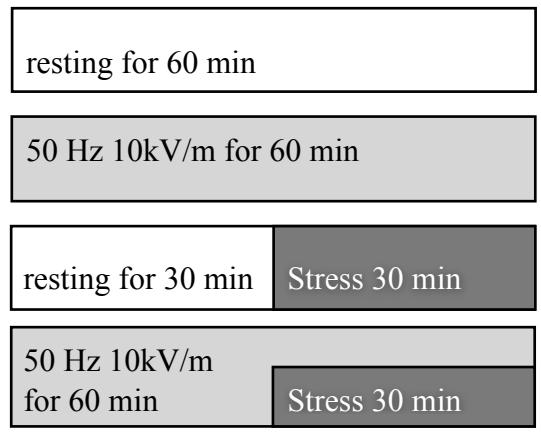
↙ ↘

Test 1

in the 3 groups regarding to gender, intact male, intact female, and ovariectomized female.

Test 2

in the 3 ages, 15, 25, and 55 weeks old




↘ ↙

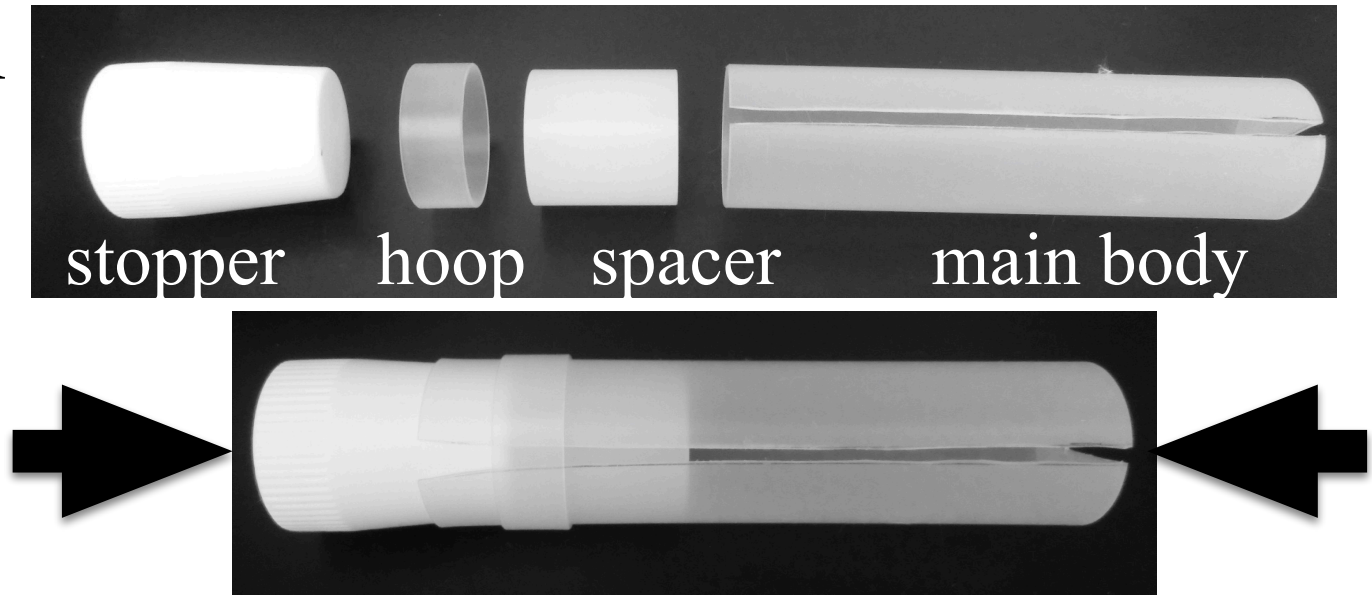
Sample collection

↙ ↘

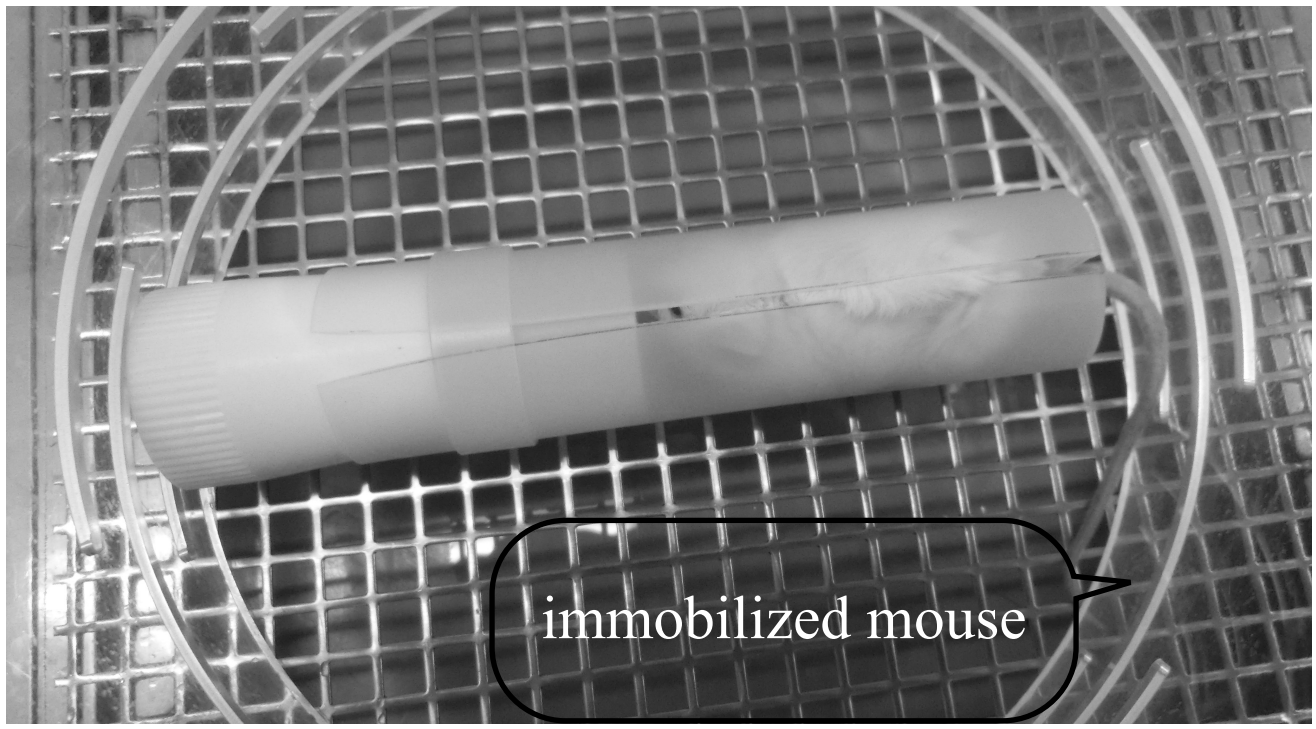
glucocorticoid measurement

blood property analysis

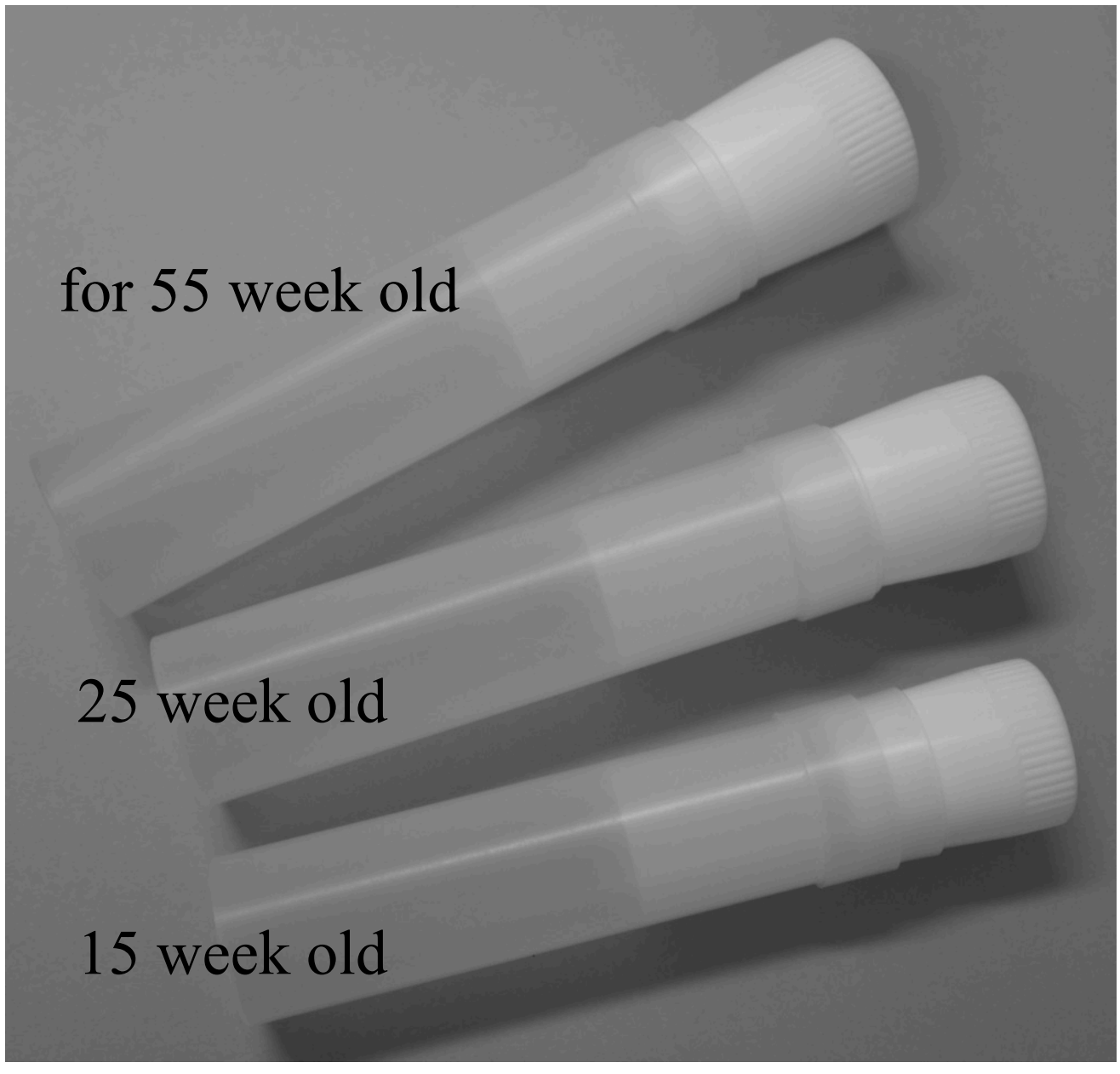
A

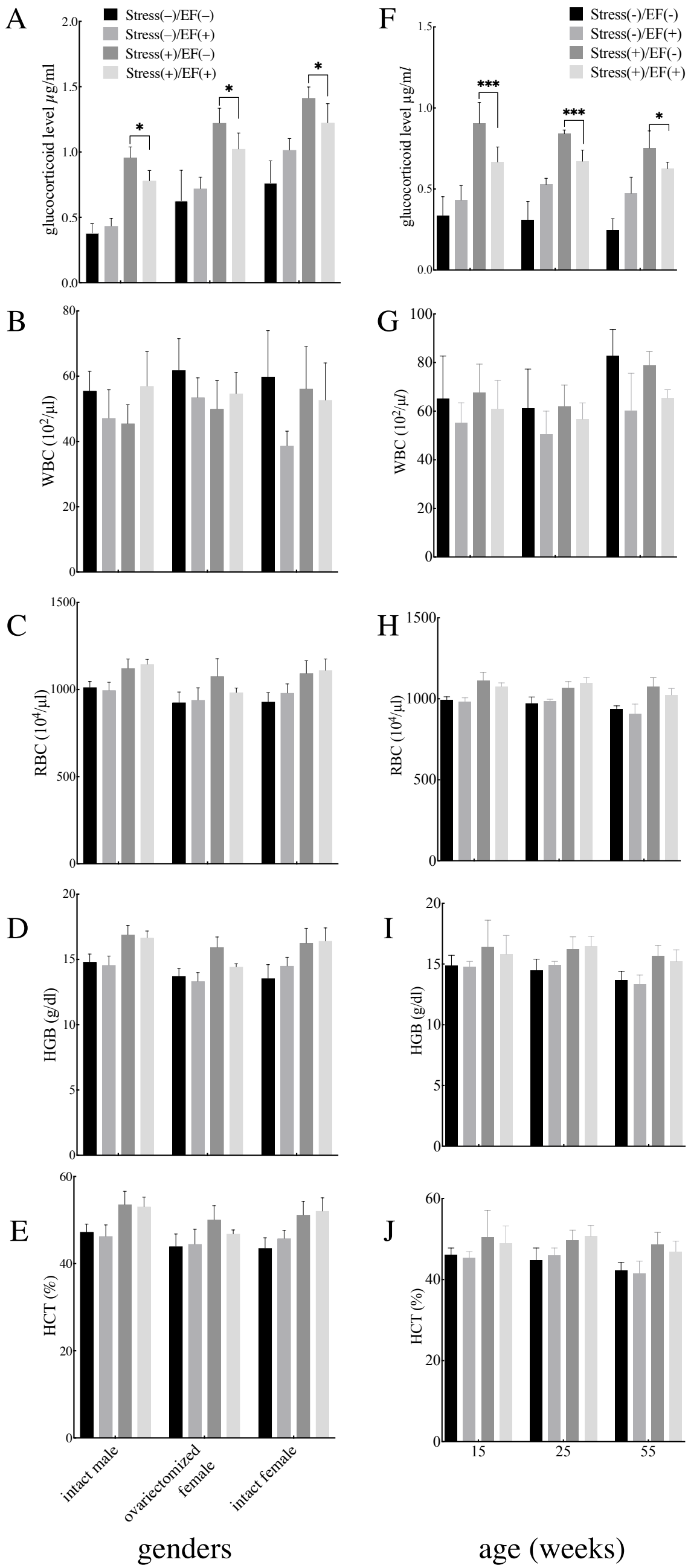

stopper
hoop
spacer
main body

B


immobilized mouse

C


for 55 week old
25 week old
15 week old


A
Stress(–)/EF(–)
Stress(–)/EF(+)
Stress(+)/EF(–)
Stress(+)/EF(+)
glucocorticoid level μg/ml
F
Stress(-)/EF(-)
Stress(-)/EF(+)
Stress(+)/EF(-)
Stress(+)/EF(+)
glucocorticoid level μg/ml
B
WBC (10²/μl)
G
WBC (10²/μl)
C
RBC (10⁴/μl)
H
RBC (10⁴/μl)
D
HGB (g/dl)
I
HGB (g/dl)
E
HCT (%)
J
HCT (%)
intact male
ovariectomized female
intact female
genders
15
25
55
age (weeks)