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Body weight and leukocyte infiltration after an acute exercise-related muscle injury in ovariectomized mice treated with estrogen and progesterone

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1. Introduction

1.1. Intramuscular leukocyte infiltration to estrogen and progesterone

After an acute skeletal muscle injury, leukocytes remove dead fibers and cellular debris, induce a secondary injury, and/or promote myofiber regeneration. Therefore, factors that influence leukocyte infiltration into injured muscle have a major impact on overall muscle recovery. Two such factors are the sex hormones, estrogen and progesterone.

For example, approximately 9–14 days of estrogen treatment attenuates intramuscular leukocyte infiltration in a rat exercise-induced skeletal muscle injury model [5;13;29–31]. In addition, exercise-induced injury studies revealed that the body weight of estrogen-treated rats was lower than that of placebo-treated or untreated rats [4;5;13;29–31]. Therefore, in the rat estrogen treatment-exercise muscle injury model, a short-term (9–14 days) treatment of estrogen simultaneously and consistently affects both body weight and leukocyte infiltration.

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In contrast, the body weight data from murine estrogen treatment studies are not as consistent as the rat estrogen treatment studies. That is, Gorzek et al. [7] and Moran et al. [18] observed that the body weight of ovariectomized-estrogen-treated mice was significantly lower than that of ovariectomized-placebo-treated mice after exercise, whereas Warren et al. [32] and Schneider et al. [22] reported that these groups had similar body weights after exercise-induced muscle injury. This intraspecies body weight difference may be related to the duration of estrogen treatment. For example, Gorzek et al. [7] and Moran et al. [18] treated the mice for ~ 4–6 weeks, which is ~ 1–4 weeks longer than Warren et al. [32] and Schneider et al. [22]. Nevertheless, the duration of estrogen treatment is unlikely to account for the interspecies difference because mice did not have a lower body weight when treated with ~ 2 weeks of estrogen as rats did with a 2-week treatment period [4;5;13;29–31]. Therefore, these data suggest that the body weight response to estrogen treatment may differ between rats and mice.

Regarding progesterone, Iqbal et al. [13] reported a 20–30% reduction in intramuscular leukocyte infiltration in ovariectomized-progesterone-treated rats in comparison with intramuscular leukocyte infiltration in ovariectomized-untreated rats. This progesterone effect was significantly smaller than that of estrogen. Interestingly, there was no body weight difference between these rats. Therefore, there is a need to increase our understanding of both estrogen and progesterone's role in the leukocyte response after an exercise-induced injury. Because there is an inconsistency in the murine body weight response to exogenous estrogen, and the absence of murine studies examining both body weight and the intramuscular leukocyte response to ovarian hormone level changes, the aim of the current study was to compare body weight and leukocyte infiltration in exercise-injured skeletal muscle of ovariectomized mice treated with exogenous estrogen and progesterone for the short period of 17 days with that of ovariectomized-placebo-treated mice and gonadal-intact male and female mice. This treatment period was selected to examine a duration that was used in a previous murine muscle injury study [32].

2. Material and methods

2.1. Animals

Thirty-six ovariectomized female, 9 gonadal-intact female, and 9 gonadal-intact male C57BL/6 mice (6–7 weeks old) were purchased from Harlan Laboratories (Indianapolis, IN). The animals were fed water and food ad libitum and housed one mouse per cage in the animal care facility with a 12-hr light/12-hr dark cycle. The use of animals in the study procedures was approved by the University of Wisconsin-Madison School of Medicine Institutional Animal Care and Use Committee.

2.2. Pellet insertion protocol

Seven days after arrival to the animal care facility, the ovariectomized mice underwent pellet insertion. First, the animals received a subcutaneous injection of buprenorphine 0.05–0.1 mg/kg. Then the animals were anesthetized using inhalation isoflurane with 100% oxygen. When the mice were nonresponsive, an area at or near the shoulder or back was prepped and one or two pellets were surgically inserted. Twenty-seven ovariectomized mice received one of the following 21-day release pellets (Innovative Research of America, Sarasota, FL): 17- β estradiol 0.05 mg pellet, progesterone 15 mg pellet, or placebo 0.05 mg pellet. The remaining 9 ovariectomized mice received both estradiol and progesterone pellets. The estrogen and progesterone doses were chosen because earlier findings indicated that these doses would produce blood estradiol and progesterone levels similar to levels present in gonadal-intact, cycling female mice [6;8;9].

2.3. Eccentric contraction muscle injury protocol

On Day 16 of hormone treatment, the ovariectomized mice underwent an eccentric contraction muscle injury (ECMI) protocol. The intact mice underwent this protocol after the same amount of total housing time as the ovariectomized mice. The ECMI protocol was based on the one developed by Ashton-Miller et al. [1]. Each mouse was anesthetized. The animal's left hindlimb was shaved and cleaned with povidone-iodine scrub, water, povidone-iodine solution, and then 70% isopropyl alcohol. The animal's left foot was strapped into a shoe, to which a servomotor (Aurora Scientific Inc.; Aurora, Ontario) was attached. The ankle was maintained at 90° and the knee was stabilized by an L-shaped bar. Two needle electrodes were placed percutaneously near the tibial nerve. Electrode placement was ascertained by delivering a single 300-msec stimulus (150 Hz, 4 volt, 0.2 msec pulse width, Harvard Dual Impedance Research Stimulator, Harvard Apparatus, Holliston, MA) to the nerve and checking for a peak isometric torque. Then the mouse underwent 3 bouts of 150 trials (total of 450 trials). A trial began with a 150 Hz, 4 volt, 0.2 msec pulse width stimulus. During the last 200 msec of the stimulus, the foot was dorsiflexed at an ankle rotation of 26°, which caused the plantarflexor muscles to stretch or eccentrically contract. The trial ended by the passive return of the foot to its original position within the next 200 msec. There was an additional 200 or 350 msec of no activity. Each trial lasted a total of 800 or 950 msec. Five-min rest periods occurred after the 150th and 300th trials (after the 1st and 2nd bouts). Nerve stimulation and dorsiflexion were controlled by SuperScope II version 2.17 or 3.00 (GW Instruments, Somerville, MA) run on a Macintosh computer (Apple Computers Inc., Cupertino, CA). The servomotor and stimulator were linked to the computer using a Model 100B instruNet network device and software (GW Instruments).

After completing the ECMI protocol, anesthesia was stopped and each mouse was returned to a cage. Of the 54 mice, only one mouse received a buprenorphine s.c. injection postECMI because of impaired ambulation.

2.4. Animal weights

The ovariectomized mice were weighed immediately after pellet insertion, and before the ECMI protocol and euthanasia. The intact mice were weighed before ECMI protocol and euthanasia.

2.5. Tissue harvest and euthanasia

The day after undergoing the ECMI protocol, the mice were anesthetized and the plantarflexor muscles were dissected. The anesthetized mice were euthanized by cervical dislocation. The uterine horns were dissected bilaterally from the intact female and ovariectomized mice and then weighed.

2.6. Preparation of muscle cross-sections

The plantarflexor muscles were frozen immediately in melting isopentane cooled by liquid nitrogen. Frozen muscles were maintained at -70 °C until sectioning. Ten-micron thick serial cross-sections from or near the midbelly of the lateral gastrocnemius were cut using a cryostat (Leica CM1850; Leica Microsystems Inc., Bannockburn, IL). Cross-sections were applied to poly-L-lysine slides and kept frozen until immunohistochemistry.

2.7. Immunohistochemistry

The primary antibody used was rat monoclonal anti-mouse 7/4 (Caltag Laboratories, South San Francisco, CA). This antibody detects neutrophils [12] and monocytes [11].

The immunohistochemistry procedure was as follows. Slides were air dried at room temperature, fixed in acetone at room temperature, and then air dried again. Sections were rinsed in phosphate buffered saline (PBS) and then blocked with 2% bovine serum albumin in PBS. After a PBS rinse, the 7/4 antibody (1:20 dilution; Caltag Laboratories) was applied for 2 hr. Sections were rinsed again in PBS and then incubated with the secondary antibody, biotinylated anti-rat IgG (mouse adsorbed; 1:200; Vector Laboratories, Burlingame, CA) for 30 min followed by a PBS rinse. Endogenous peroxidase was quenched by immersing all sections in 0.3% hydrogen peroxide in methanol. All sections were rinsed in PBS, incubated with Vectastain ABC peroxidase reagent (Vector Laboratories) for 30 min, and then rinsed again. A diaminobenzidine substrate/nickel chloride solution (Vectastain DAB substrate kit, Vector Laboratories) was applied to all sections for 5 min and the substrate reaction was stopped by immersing the sections in distilled water. After a water wash, the sections were dehydrated with 95% and 100% ethanol, cleared with Hemo De (Scientific Safety Solvents, Keller, TX), air dried, cleared with Hemo De, and mounted with a coverslip using the nonaqueous medium, Cytoseal (VWR Scientific, Batavia, IL). Control slides were generated by omitting the primary antibody step of the immunohistochemistry procedure. After the slides dried, each slide was coded by applying a numbered piece of tape over any identifying information marked on the slide.

2.8. 7/4 antibody labeling image analysis

7/4 antibody labeling analysis was performed using a computer-assisted approach. Thirty-six-RGB bit images were captured using a 10 × objective (100 × magnification) attached to an Eclipse E600 light microscope (Nikon Inc., Melville, NY), a SPOT RT KE color mosaic camera (Diagnostics Instruments Inc., Sterling Heights, MI), and Image-Pro® Plus 5.1.2 software (Media Cybernetics Inc., Silver Spring, MD) run on a Dell OptiPlex GX280 computer (Round Rock, TX) with a 1905FP Dell monitor. Each image represented an area of interest (AOI) within a consistent region of the eccentrically-contracted lateral gastrocnemius muscle. One AOI (1.02 mm²) was generated for each mouse. Image preparation and computer pixel assignment was performed similarly as described in a previous publication [25]. Image preparation included computer enhancements and manual erasing to remove false-positive staining, debris, holes, and large connective tissue areas. This erasing was double-checked by a different co-author and yielded an adjusted AOI ranging in area, 0.74–1.01 mm². The majority of these adjusted AOI images were used in an earlier study to examine the reliability between manual and computer-assisted counts [25]. However, in the earlier report only the 7/4 leukocyte number data were used but were not presented. Additionally, AOI images from control slides were captured, and these images matched the 7/4 images. These control sections were not more than three sections away from the 7/4 sections.

For each adjusted AOI, four measures were completed: number of 7/4 leukocytes, 7/4 leukocyte area percentage, mean 7/4 antigen area, and frequency of 7/4 cell area ranges. The number of 7/4 leukocytes corresponded to the number of objects identified by the Image-Pro® Plus 5.1.2 software at an operator-selected grayscale value. The grayscale value was based on using 1 to 3 preselected objects with medium immunolabeling within the AOI. This grayscale value was verified by a second observer. The 7/4 leukocyte area percentage was determined by converting the 7/4 antibody-labeled pixels to area, dividing this labeled area by the adjusted AOI area, and then multiplying by 100. The mean 7/4 antigen area was determined by calculating the mean of individual object areas within an adjusted AOI (Fig. 1). The frequency of 7/4 cell area ranges was calculated by counting the number of cells that fall within the area ranges, 7–12, 13–24, 25–49, and 50–206 μm². These ranges were used because ~ 25% of the cell areas from all animal groups fall within 7–12 μm², ~ 50% within 7–24 μm², ~ 75% within 7–49 μm², and 97.5% at ≤ 206 μm².

2.9. Statistical analysis

Data are expressed as means $\pm$ SE, except the frequencies of 7/4 cell area ranges are stated as actual counts. Data were analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC) or SPSS version 16 (SPSS Inc., Chicago, IL). Mean differences between groups were analyzed using analysis of variance. For significant tests, post-hoc analysis was performed using Bonferroni (Dunn) *t* tests. The frequency of 7/4 cell area ranges between groups were analyzed using a likelihood ratio test. Alpha was set at .05 for all statistical tests.

3. Results

3.1. Weights

Table 1 shows (a) body and (b) absolute and relative uterus horn weights. On the day of pellet insertion, the body weight of the ovariectomized mice in the four groups was similar ($F = 0.23$, $p = .88$). On the day of ECMI and euthanasia, the body weight of the ovariectomized-estrogen-progesterone-treated and intact male groups was significantly greater than the ovariectomized-placebo-treated, ovariectomized-progesterone-treated, and intact female groups ($p < .05$). Although the body weight of the ovariectomized-estrogen-treated group increased 18% from pellet insertion to eccentric contractions and the body weight of the ovariectomized-placebo-treated group only rose 7% during the same time period, there was no significant difference in the body weight between these two groups. The ovariectomized-estrogen-treated group had the highest relative uterus horn weight, whereas ovariectomized-placebo-treated and ovariectomized-progesterone-treated had the lowest weight of all the groups ($p < .05$).

3.2. 7/4 antibody labeling

One day after eccentric contractions, 7/4 antibody labeling was present in the specified area of the lateral gastrocnemius in 47 of 54 (87%) mice. Most of the labeling was observed in connective tissue areas. For certain mice, labeling was also present within muscle fibers. The 7/4 leukocyte number (Fig. 2) and area percentage (Fig. 3) did not significantly differ among hormone treatment groups. In contrast, a significant difference in mean 7/4 antigen area was detected (Fig. 4). The mean 7/4 antigen area of the ovariectomized-estrogen-treated group was 2.2–3.4-fold greater than that of the ovariectomized-placebo-treated, ovariectomized-estrogen-progesterone-treated, and intact male groups. Additionally, there was a significant difference in the proportion of size distribution by hormone group (Fig. 5). More 7/4 cells tended to be larger in the ovariectomized-estrogen-treated group than the other groups, and the 7/4 cells tended to be smaller in the intact male group.

4. Discussion

4.1. Overview

The aim of the current study was to compare body weight and leukocyte infiltration in exercise-injured skeletal muscle of ovariectomized mice treated with exogenous estrogen and progesterone for the short period of 17 days with that of ovariectomized-placebo-treated mice and gonadal-intact male and female mice. There were two major findings. The body weight of ovariectomized-estrogen-treated mice was not significantly different from that of ovariectomized-placebo-treated mice. In addition, the most novel finding is that the mean 7/4 antigen area of the ovariectomized-estrogen-treated group was 2–3-fold greater than that of the ovariectomized-placebo-treated, ovariectomized-estrogen-progesterone-treated, and intact male groups, suggesting that the 7/4 leukocytes of the ovariectomized-estrogen-treated group were larger or had more antigen. This finding was corroborated by a likelihood ratio test that indicated larger cells or cells with more antigen were present in the ovariectomized-estrogen-treated group.

4.2.1. Species difference in body weight—Our and others' finding [22;32] of similar body weights between ovariectomized-estrogen-treated (for a period of 2.5–3 weeks) and ovariectomized-placebo-treated mice contrasts with that of other studies in which murine body weight was significantly lower with 4 or more weeks of estrogen treatment [7;18]. But recently, Witte et al. [33] showed that ovariectomized mice exhibited sustained weight gain, starting at 14 days postsurgery. Taken together, these current and previous data suggest that estrogen may interfere with weight gain in ovariectomized mice when administered for longer than 3 weeks.

The duration of estrogen treatment needed to hinder body weight gain in ovariectomized mice contrasts with the duration used with ovariectomized rats. In rats, estrogen administration for only about 1.5–2 weeks prevented weight gain [4;5;13;29–31]. But this species difference may be explained by the finding that ovariectomized rats demonstrate sustained increased food intake between 3 and 21 days postsurgery, unlike ovariectomized mice [33]. Therefore, short-term estrogen administration may counteract food intake induced by ovariectomy in rats, but not in mice.

Alternatively, age at ovariectomy may influence murine body weight. In the Gorzek et al. [7], Moran et al. [18], and Witte et al. [33] studies, the mice were ovariectomized at 3–4 months of age, whereas the mice were ovariectomized at 1–2 months of age in the Warren et al. [32] and Schneider et al. [22] studies. However, mice ovariectomized at approximately 3.5 months and treated with estrogen for only 2 weeks exhibited a similar body weight as ovariectomized-placebo-treated mice [10]. Taken together, these data suggest that the age at ovariectomy is unlikely to account for the conflicting effects of exogenous estrogen on body weight of ovariectomized mice.

Additionally, age at ovariectomy does not explain the species difference in the body weight response to short-term estrogen treatment. Both rats [4;5;13;26;29–31] and mice [22;32] were ovariectomized at about 1–2 months of age. In conclusion, the species difference in estrogen's body weight effect is more likely due to the duration of the estrogen treatment than age at ovariectomy. Future studies will need to test the hypothesis that long-term estrogen treatment prevents weight gain in ovariectomized mice.

4.2.2. Species difference in leukocyte infiltration after muscle injury—A species difference in response to short-term estrogen administration may also be evident with intramuscular leukocyte infiltration. In the current study, a difference in leukocyte number or area percentage between the ovariectomized-estrogen-treated and ovariectomized-placebo-treated groups and between the ovariectomized-progesterone-treated and ovariectomized-placebo-treated groups was not detected and conflicts with previous data. In earlier studies [5;13;29–31], the leukocyte (neutrophil and macrophage) number of the ovariectomized-estrogen-treated or ovariectomized-progesterone-treated group was significantly lower than that of the control group. It is difficult to speculate a possible explanation for this species difference because the specific mechanism by which estrogen and progesterone attenuates intramuscular leukocyte infiltration has not been determined. Based on the species difference in body weight change to ovariectomy observed in the exercise-induced muscle injury studies [5;13;22;29–32], the estrogen treatment period appears to differentially affect body weight change in these rodent investigations. Similarly, the estrogen treatment period may differentially affect intramuscular leukocyte infiltration. For example, more than 2.5 weeks of estrogen treatment may be required to attenuate intramuscular leukocyte infiltration in ovariectomized mice, whereas only 1.5–2 weeks of estrogen treatment may be needed to attenuate intramuscular leukocyte infiltration in ovariectomized rats and gonadal-intact rats.

4.3. 7/4 antigen area

The most novel finding of the current study is that the mean 7/4 antigen area of the ovariectomized-estrogen-treated group was 2–3-fold greater than that of the ovariectomized-placebo-treated, ovariectomized-estrogen-progesterone-treated, and intact male groups. Described in 1983, the 7/4 antigen was originally detected exclusively on blood, bone marrow, and peritoneal neutrophils of certain mouse strains without any age or sex differences [12]. Then in 2003, the 7/4 antigen was detected on monocytes [11;28]. Seventeen years after its original detection, the 7/4 antigen has been identified as a Ly-6B protein connected to the cell membrane via a glycosylphosphatidylinositol-anchor and has been detected on other cells, including mature macrophages [21].

The identification of the 7/4 antigen as a Ly-6 protein ties this antigen to the family of Ly-6-genes located on chromosome 15 [21]. Despite this recent identification, the function of this antigen for neutrophils, monocytes, or macrophages remains unknown. To date, the 7/4 antigen has been primarily used as a marker to detect these cells in blood, peritoneal fluid, bone marrow, and other tissue, and has been consistently detected after chemical, inflammatory, or injurious stimuli in different tissues, including skeletal muscle [2;16;17;23].

The finding of greater mean 7/4 antigen area in the injured muscle of ovariectomized-estrogen-treated mice suggests that estrogen may modulate the 7/4 antigen. Although Hirsch and Gordon [12] originally reported that antigen expression was not sex-related, these investigators used a different method to determine sex differences than the one used in the current study. Other studies showing estrogen modulation of the 7/4 antigen are lacking. However, exogenous estrogen increases the mRNA expression of the Ly-6 gene, RoBo-1, in the bone of ovariectomized rats [20], suggesting that certain Ly-6 molecules are responsive to exogenous estrogen.

Furthermore, for certain variables, the combination of estrogen and progesterone in ovariectomized animals yields a response similar to that of estrogen alone. Examples include murine macrophage C3b receptor expression, phagocytosis, and oxidative metabolism [3], murine time-to-muscle-fatigue [22], and rat neutrophil and macrophage infiltration into exercise-injured muscle [13]. This response pattern differs from the current investigation in which the mean 7/4 antigen area of the OEP group was significantly different from that of the OE group and the mean 7/4 antigen area of the OEP and OP groups was similar. These current findings suggest that (a) progesterone has no direct effect on mean 7/4 antigen area and (b) progesterone must be absent for estrogen to influence the mean 7/4 antigen area.

The current study also broadens our understanding of potential sex or sex hormone differences in the leukocyte response to injured muscle. Previously, sex or sex hormone differences in the timing and number of leukocytes has been the experimental focus of the leukocyte response to exercise induced muscle injury [5;13;24;27;29–31]. In light of the current mean 7/4 antigen area data, estrogen may also modulate leukocyte antigen amount or phenotype. Findings from three studies support this possibility. Lamote et al. [15] observed reduced CD47 expression after blood bovine neutrophils were treated with estrogen for 3 h. Mossuz et al. [19] reported an upregulation of CD13 and CD64 antigens after human monoblastic leukemic cells, U937, were exposed to estrogen. Kramer et al. [14] showed estrogen downregulation of human monocyte CD16 expression.

Furthermore, Kramer et al. [14] reported that estrogen's CD16 downregulation led to a reduction in human monocyte interleukin-6, interleukin-1 β , and tumor necrosis factor production. This result suggests that estrogen may modulate leukocyte function as well and

provides a basis for additional exercise muscle injury studies to investigate whether estrogen related-increased 7/4 leukocyte expression influences muscle recovery processes.

4.4. Conclusions

Two major conclusions can be drawn from this current study. One conclusion is that ovariectomized mice demonstrate different body weight and muscle inflammatory responses to short-term estrogen treatment than ovariectomized-estrogen-treated rats. Specifically, the findings of this study suggest that body weight and amount of intramuscular leukocyte infiltration of ovariectomized mice treated with short-term estrogen are similar to that of ovariectomized-placebo-treated mice in contrast to previous ovariectomized rat data.

A second conclusion of the present investigation is that short-term estrogen treatment modulates leukocyte phenotype. The results suggest that estrogen increases the amount of 7/4 antigen, an antigen that appears in leukocytes infiltrating injured muscle. Future research will address whether this modulation influences muscle recovery processes.

Highlights

Estrogen has no effect on body weight.

The 7/4 leukocyte number and area percentage was unaffected by ovarian hormones.

Mean 7/4 antigen area was greater in the ovariectomized-estrogen-treated group.

Abbreviations

ECMI	eccentric contraction muscle injury
AOI	area of interest

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C

Object #	7/4 Antigen Area (μm^2)
1	9
2	42
3	38
4	46
5	8
6	22
7	19
8	18
9	48
10	12
11	38
12	25
Mean	27

Fig. 1.

A. Portion of a lateral gastrocnemius muscle cross-section stained with hematoxylin and eosin. This muscle underwent 450 eccentric contractions. The image shows muscles fibers and nuclei of muscles fibers and other cells present in the connective tissue. B. Mean 7/4 antigen area method. This image shows the identification of 12 (black) objects by the Image-Pro[®] Plus 5.1.2 software within an AOI. A 36-bit RGB image was converted to a grayscale value and assigned a threshold to apply pixels to identify the 7/4 labeling within the image. These black objects represent positive 7/4 antibody labeling. C. Examples of 7/4 antigen area. Image-Pro[®] Plus software can calculate each object's μm^2 , which represents the object's 7/4 antigen area. The mean 7/4 antigen area is calculated by finding the object area

mean per AOI. The mean 7/4 antigen area for this AOI is $27 \mu\text{m}^2$. *Abbreviation:* AOI, area of interest.

Fig. 2. 7/4 leukocyte number. There was no significant difference among the groups ($F = .91$; $p = 0.485$; $n = 9$ per group). Group abbreviations are the same as listed in Table 1.

Fig. 3. 7/4 leukocyte area percentage. The area percentage of 7/4 antibody labeling was similar among the groups ($F = 1.90$; $p = 0.112$, $n = 9$ per group). Group abbreviations are the same as listed in Table 1.

Fig. 4. Mean 7/4 antigen area. Mean 7/4 antigen area was significantly different between the groups ($F = 4.80$; $p = .0012$, $n = 9$ per group). The mean 7/4 antigen area was significantly greater in the OE group than in the OC, OEP and IM groups ($*p < .05$). Group abbreviations are the same as listed in Table 1.

Fig. 5.

Cell area and frequency. (A) Cell area ranges. Examples of cell areas within the ranges of 7–12 μm^2 , 13–24 μm^2 , 25–49 μm^2 , and 50–206 μm^2 : a = 24; b = 40; c = 50; d = 144; e = 10; f = 26. (B) Cell frequency by hormone treatment. The likelihood ratio was 147.78 ($p < .05$). □ OP ($n = 8$); ▨ IF ($n = 9$); ■ OE ($n = 9$); ▩ OEP ($n = 8$); ▤ OC ($n = 7$); ■ IM ($n = 6$). Group abbreviations are the same as in Table 1.

Table 1

Body and absolute and relative uterus horn weights

	BWP, g	BWEC, g	BWEU, g	UH, mg	UH/BW, mg/g
IM (<i>n</i> = 9)	Not Done	25.92 ± 0.48	25.30 ± 0.46	None	N/A
IF (<i>n</i> = 9)	Not Done	21.45 ± 0.48	20.88 ± 0.50	0.07 ± 0.01	3.53 ± 0.46
OC (<i>n</i> = 9)	21.15 ± 0.48	22.63 ± 0.53	22.15 ± 0.45	0.01 ± 0.00	0.45 ± 0.04
OE (<i>n</i> = 9)	20.88 ± 0.45	24.54 ± 0.34	23.99 ± 0.37	0.13 ± 0.01	5.61 ± 0.28
OP (<i>n</i> = 9)	21.15 ± 0.30	22.81 ± 0.50	22.51 ± 0.50	0.01 ± 0.00	0.57 ± 0.07
OEP (<i>n</i> = 9)	21.39 ± 0.49	25.89 ± 0.51	25.53 ± 0.51	0.07 ± 0.00	2.72 ± 0.12

Abbreviations: IM, gonadal-intact male; IF, gonadal-intact female; OC, ovariectomized female mice treated with placebo pellet; OE, ovariectomized female mice treated with estradiol pellet; OP, ovariectomized female mice treated with progesterone pellet; OEP, ovariectomized female mice treated with estradiol and progesterone pellets; BWP, body weight at hormone pellet insertion; BWEC, body weight before the eccentric contraction injury protocol; BWEU, body weight at euthanasia; UH, absolute uterus weight; UH/BW, relative uterus horn weight, absolute uterus horn weight divided by animal's body weight at euthanasia, multiplied by 1000. *Note:* Values are means ± SE.