HHS Public Access

Author manuscript

J Toxicol Environ Health A. Author manuscript; available in PMC 2023 March 02.

Published in final edited form as:

J Toxicol Environ Health A. 2022 December 02; 85(23): 953–971. doi:10.1080/15287394.2022.2128954.

Effects of whole-body vibration on reproductive physiology in a rat model of whole-body vibration

K. Krajnak,

S. Waugh,

D. Welcome,

X.S. Xu,

C. Warren,

W. McKinney,

R.G. Dong

Physical Effects Research Branch, National Institute for Occupational Safety and Health, Morgantown, West Virginia

Abstract

Findings from epidemiological studies suggest that occupational exposure to whole-body vibration (WBV) may increase the risk of miscarriage and contribute to a reduction in fertility rates in both men and women. However, workers exposed to WBV may also be exposed to other risk factors that contribute to reproductive dysfunction. The goal of this experiment was to examine the effects of WBV on reproductive physiology in a rat model. Male and female rats were exposed to WBV at the resonant frequency of the torso (31.5 Hz, 0.3 g amplitude) for 4 hr/day for 10 days. WBV exposure resulted in a significant reduction in number of developing follicles, and decrease in circulating estradiol concentrations, ovarian luteinizing hormone receptor protein levels, and marked changes in transcript levels for several factors involved in follicular development, cell cycle, and steroidogenesis. In males, WBV resulted in a significant reduction in spermatids and circulating prolactin levels, elevation in number of males having higher circulating testosterone concentrations, and marked alterations in levels of transcripts associated with oxidative stress, inflammation, and factors involved in regulating the cell cycle. Based upon these findings data indicate that occupational exposure to WBV contributes to adverse alterations in reproductive physiology in both genders that may lead to reduction in fertility.

CONTACT K. Krajnak, ksk1@cdc.gov, Physical Effects Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Rd, Morgantown, West Virginia 26505.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Mention of brand name does not constitute product endorsement.

Keywords

Whole body vibration; endocrine; reproduction; hormone levels; gene transcription

Introduction

Workers in many sectors including Transportation, Warehousing and Utilities, Construction, Agriculture, Forestry, Fisheries, and Mining are regularly exposed to whole-body vibration (WBV) while driving large transportation or earth-moving vehicles or while using large vibrating tools such as chain saws or rock drills. Exposure to WBV has been associated with an increased risk for neck and back pain (Charles et al. 2018; Du et al. 2018; Miyashita et al. 1992; Morgan and Mansfield 2014). Further the findings of other studies suggested that occupational exposure to WBV may also serve as a risk factor for the development of several diseases including cardiovascular diseases and cancer (Nadalin et al. 2012; Pukkala et al. 2009; Wang et al. 2021; Waugh et al. 2016; Young et al. 2009).

There also are epidemiological studies demonstrating that exposure to WBV may increase the risk of pre-term birth (Croteau, Marcoux, and Brisson 2007; Hartikainen et al. 1994; Skroder et al. 2020, 2021), result in fertility problems in both men and women (Abdollahi et al. 2021), as well as diminished sperm motility and sperm count (Zarie et al. 2022). There has been a rise in the number of women working in positions where they are exposed to WBV in recent years (U.S. Bureau of Labor Statistics 2011, 2021). Because the morphometry of the torso is different in men and women, and these differences may affect the transmission of vibration throughout the body (Eng et al. 2011; Govers et al. 2021; Milanese et al. 2012), the risk of developing specific vibration-induced injuries or diseases may vary between men and women. In addition, differences in other physiological factors, such as (1) circulating hormone concentrations (Ferrell, Giza, and Shibao 2020), (2) distribution of specific receptors that regulate blood flow (Eid et al. 2007; Jeyaraj et al. 2012), or (3) peripheral sensory function (Liou et al. 1999), may also affect responsiveness to vibration. Therefore, examining the effects of WBV exposure in both males and females, and determining the roles played by various physical and physiological factors in the actions of WBV on health outcomes is important such that standards may be written, and interventions developed to protect the greatest number of workers.

Workers exposed to WBV are also often exposed to other factors that affect health such as toxic fumes (Eng et al. 2011; Jungwirth et al. 1997; Pukkala et al. 2009; Rushton et al. 2010), unusual work schedules (Diez et al. 2020; Stoynev and Minkova 1997), and awkward postures (Jonsson et al. 2015; Scott and Stout 2013). It is not clear if all these factors contribute equally to the risk of a worker developing adverse health problems, or if some factors pose a greater risk. Because most workers are exposed to multiple risk factors at one time, understanding the health effects of each factor separately, and then, how these may act together to affect worker health, is critical if vehicles or tools are going to be designed to reduce exposure to the factors that pose the greatest risk for affecting workers health.

In the current study, a rat model of WBV was developed and characterized to begin to understand the role that this specific work-related parameters may play in affecting

health. In preliminary studies, the response of the body (head and torso) to WBV was measured in both male and female rats to determine the resonant frequency. Previously, investigators demonstrated that exposure to vibration at the resonant frequency induces the greatest tissue stress and strain, resulting in more tissue damage and disruptions in physiological functioning more quickly than exposure at other frequencies (Krajnak et al. 2010, 2012a; Dong et al. 2012; Griffin, Bovenzi, and Nelson 2003; Bovenzi 2002; Wang et al. 2010; Milanese et al. 2012). Therefore, based upon the results of previous studies, animals were exposed to vibration at the resonant frequency of the torso and the effects on reproductive/neuroendocrine function were assessed. It was hypothesized that repetitive exposure to WBV at the resonant frequency (31.5 Hz females and 20–30 Hz males) of the torso would disrupt reproductive/neuroendocrine physiology in both males and females. Previously several investigators demonstrated that exposure to vibration at the resonant frequency resulted in (1) greater levels of oxidative stress, (2) reductions in blood flow, and (3) changes in the expression of transcripts that regulate cell cycle (Krajnak et al. 2010, 2012b, 2009; Waugh et al. 2016). These factors were measured in reproductive organs to determine if similar changes might be associated with health/reproductive-related alterations after exposure to WBV at or near the resonant frequency of the lower lumbar region. The influence of exposure to WBV was also examined on circulating concentrations of both gonadal and pituitary steroids. Changes in circulating concentrations of these hormones and pathways activated by these hormones may serve as early biomarkers of WBV-induced effects on reproductive function and general health.

Methods

Animals

Male (n = 12) and female (n = 12) Sprague-Dawley rats (H1a: (SD) CVF, approximate body weight of 200–230 g at arrival), were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA). All animals were free of viral pathogens, parasites, mycoplasm, *Heliobacter*, and cilia-associated respiratory bacillus. Upon arrival, animals were acclimated to AAALAC International accredited animal facilities at NIOSH for one week. Rats were housed in ventilated micro-isolator units supplied with HEPA-filtered laminar flow air (Lab Products OneCage; Seaford, DE), Teklad Sanichip and Shepherd Specialty Paper's Alpha-Dri cellulose, tap water, and autoclaved Teklad rodent diet (Harlan Teklad; Madison, WI) *ad libitum*. Animals were housed in same-sex pairs, and under a controlled 12 hr light/dark cycle and temperature (22–25°C) conditions.

One week following acclimation, animals were randomly assigned to control or WBV conditions. The exposure and all other procedures performed were approved by the Institutional Animal Care and Use Committee and in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.

Equipment and characterization of the exposure

Platforms (L 20.32 cm × W 15.24 cm × thickness 1 cm) were printed on a 3D printer (Fusion3 F410) using a poly lactic acid polymer. Flat bottomed restrainers were purchased

(Braintree Scientific, Braintree, MA; dimensions inside the restrainer L 22 cm × W 8.26 cm × height at center 6.35 cm) and the NIOSH machine shop removed the bottom and added an extension to the side of each restrainer so that it could be attached to the platform. Platforms were attached to the shakers, and a restrainer was attached to each platform. An accelerometer (353B15, PCB Piezotonic, DePew, NY) was attached to the middle of the platform, next to the restrainer (see Figure 1). The restrainer, platform, and shaker were all placed in a sound-attenuating chamber. Vibration to the platform was generated by a shaker (Ling Dynamics, Royston, UK). To ensure there were no resonances of the platform in the frequency range of concern (< 100 Hz), a laser vibrometer was used to measure both the response of the restrainer and the platform between 10 and 100 Hz at 0.3 g. The platform and restrainer were stable up to 100 Hz. Control animals were placed in Broome Style restrainers and housed in sound-attenuating chambers during the restraint-only (control) exposure.

Exposure

After acclimation to the facilities, animals were acclimated to restraint for 5 days. Acclimation to restraint consisted of placing animals in a Broom Style restrainer, or into a restrainer mounted onto a platform and shaker, for gradually longer lengths of time until the total time in the restrainer was 4 hr. The restrainers were large enough so that animals could move and turn around, but could not rear up onto their hind legs, thus ensuring that the animals' paws and torso were in contact with the vibrating platform or stable bottom of the restrainer, throughout the exposure. After acclimation to restraint and demonstration of the presence of normal reproductive cycles in females, exposures were started. Prior to the beginning of the experiment, the number of decibels (dB) within the exposure chamber was measured while the shakers were running. The shakers made approximately 70 dB of noise, which is below the 80 dB limit set by OSHA. Rats also can not hear below 250 Hz, and thus it is unlikely that the sound of the shakers bothered them (Koch, Gaese, and Nowatny 2022). Animals were placed into their restrainers and rats in the WBV group were exposed to vibration at 31.5 Hz and an amplitude of 0.3 g for 4 hr/day for 10 consecutive days. This frequency was selected because 31.5 Hz is near the resonant frequency of the lumbar region of the spine in female rats. This frequency was also near the range of the resonant frequency of the lumbar spine region (between 20–30 Hz) in male rats. Control animals were placed into a non-vibrating restrainer for the length of the exposure of 4 hr each day. All animals were exposed to vibration or control conditions, during the light phase of the cycle. During the exposure, animals were checked every 15 min, and most were asleep or sitting quietly during the exposure, so we don't think the time of exposure affected them. In addition, previous work done with our rat-model of hand-transmitted vibration suggested there were no circadian effects on measures other than those associated with vibration exposure. Pre-exposure body weights were collected on days 1, 5 and 10 of the experiment. Body weights were also collected immediately following the exposure on days 5 and 10 to determine if exposure induced a transient reduction in body weight. Following each exposure, animals were returned to their home cage in the animal facility.

Monitoring reproductive (estrous) cycles in females

Estrous cycles were monitored in females by performing vaginal lavage and then recording the primary cell type in the lavage fluid. While animals were being acclimated to restraint vaginal lavage was performed each day, before placing an animal in the restrainer, using previously described methods (Cora, Kooistra, and Travlos 2015; Krajnak, Rosewell, and Wise 2001). The lavage fluid was placed on a clean slide that was divided into 12 sections using a wax pencil. The fluid from an individual animal was placed in a single square on the slide. After all samples were collected, the slide was dried on a slide warmer and stained with toluidine blue. Samples were examined using a Leica microscope (Deerfield, IL) at 20 × magnification. The day of the estrous cycle was determined as described in (Cora, Kooistra, and Travlos 2015); diestrus Days 1 and 2: primarily leukocytes, proestrus: primarily nucleated cells, and estrous: primarily cornified cells. Cycles were monitored for 10 consecutive days prior to beginning the exposure. All females displayed two 4-day cycles prior to beginning the exposure. Estrous cycles were also monitored while animals were being exposed. Lavage fluid was collected daily, prior to exposing the animals to control or WBV conditions.

Tissue collection

The day after the last exposure, rats were euthanized by injection of pentobarbital euthanasia solution (100–300 mg/kg, i.p.) and exsanguinated by cardiac puncture. Blood samples were collected, allowed to clot, and centrifuged at 1200 g for 15 min at 4°C. Serum was pipetted into tubes and stored at −20°C until assayed for various hormones by ELISA. Other tissues were collected to measure vibration-induced changes in oxidative stress and RNA concentrations. The tissues, including pituitary, left ovary and testes, uteri, and prostate were extracted, placed in cryotubes, and stored at −80°C until analyzed. The right ovary and testis were collected, frozen in Tissue-Tek OTC compound (Fisher Scientific), and stored at −80°C until sectioned for histological analyses.

Oxidative stress

Tissues were homogenized in 1 ml 0.1 M phosphate-buffered saline (0.1 M PBS) containing protease inhibitors (Complete ULTRA protease inhibitor, ThermoFisher, Waltham, MA) using a bead beater. The samples were then centrifuged at 15000 g for 15 min at 4°C. The supernatant was removed and stored for protein analyses. The pellet was reconstituted in 500 μl PBS, vortexed, and stored on ice. ROS levels were measured using, 2′7′-dichlorofluorescien diacetate (DCFH-DA; Sigma-Aldrich). Duplicates of the supernatant from each pellet (10 μl) were pipetted into a 96-well plate. DCFH-DA was diluted at 1:20 in PBS (final concentration 1 mM) and 50 μl added per well. Plates were incubated in the dark for 45 min and then fluorescence measured at 490–540 nM using a Synergy H1 All in One microplate reader and Gen 5 Software package (Biotek; Winooski, VT). Background measures (wells with dye plus PBS) were subtracted from each sample, and fluorescence/μg tissue was analyzed as described below.

Western blot analyses

Western blots were performed in a manner similar to that described in (Pacurari, Waugh, and Krajnak 2019). Samples were prepared by adding a 1:1 volume of Laemelli solution to each sample, heating at approximately 50°C for 5 min, and then cooling on ice. A molecular weight ladder (Bio-rad, Irvine CA) and 10 μl (10 μg) of each sample were added to a well of a pre-cast 12% acrylamide gel (BioRad, Irvine CA). Samples were run at 200 V for approximately 1 hr or until the dye front reached the bottom of the gel. Separated proteins were then electrophoretically transferred from the gel to a nitrocellulose membrane using a mini-gel transfer system (Biorad). Membranes were rinsed in 0.1 M Tris buffered saline (TBS) and incubated in Blocking Reagent (BioRad) for 5 min at room temperature (RT). After blocking, membranes were incubated in primary antibody diluted in blocking reagent at 4°C overnight with agitation. The following morning, blots were rinsed in 0.1 M Tris-buffered saline (TBS) for 3 × 5 min and then incubated in the appropriately labeled infra-red antibody (anti-mouse or anti-rabbit; 1:5000, LiCor, Lincoln, NB) in TBS-0.3% Triton-X 100 for 1 hr at RT with agitation. Blots were then rinsed, stored in the dark at 4°C until dry, scanned using a LiCor Scanner and Odyssey Software, and the band area and density analyzed using ImageJ (National Institutes of Health; Bethesda, MD).

ELISAs.—ELISAs were used to measure circulating estradiol (E-Lab Sciences, Houston Tx), follicle-stimulating hormone, and prolactin (ALPCO, Salem, NH) in serum collected from females. In males, ELISAs for pituitary hormones were also performed, but instead of measuring estradiol, testosterone concentrations (E-Lab Sciences) were measured. Each ELISA was performed as described in the manufacturer's protocol. All samples were run in duplicate, and coefficients of variation calculated for each assay. Coefficients of variation were 12% or less for samples with either high or low concentrations of each of the hormones measured.

Tissue preparation for histology and immunohistochemistry

One ovary and one testis from each animal were frozen on dry ice, sections (20 μm) cut on a cryostat and thaw mounted onto charged slides (Fisherbrand Superfrost Plus, Pittsburgh, PA). To ensure that each slide contained sections throughout the range of the dissected tissue, the first section was placed on slide one, the second on slide two, and so on until sections had been placed on 20 slides. Then the process was repeated until there were 4–6 sections on each slide. Using this procedure, each section on a single slide was approximately 200 μm apart. Sections with slides were air dried, placed in slide boxes and stored at −80°C. One set of sections was also processed using hematoxylin and eosin (H&E) stain and the others were used for immunohistological identification of specific proteins.

H&E and immunohistochemical staining

Harris H&E staining was performed on a single slide from each animal (Krajnak et al. 2012b, 2009). Slides were thawed and fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline prior to staining. In the ovaries, the number of developing follicles was counted in each section of the ovary at a total magnification of 100X using a Leica DMLB microscope (Leica, Deerfield, IL). The average number of follicles per section

was calculated and used for analyses. In males, the number of Sertoli cells, Leydig cells, and spermatids were counted in each section using a stereological technique similar to that described in (Krajnak et al. 2006). A field was randomly chosen and a 1 mm square grid with 100 μm divisions was placed over the field of view. The number of spermatids, Sertoli cells, and Leydig cells that were within the boundaries of the grid was counted. This procedure was repeated 4 times in tissue sections from each animal. The average number of spermatids, Sertoli cells, and Leydig cells was calculated for each animal and these averages were used for analyses.

Immunohistochemistry for nitrotyrosine, estrogen receptor (ERα) and ERβ receptors, and luteinizing hormone receptor (LHR) were performed in a single set of sections from the ovary, and androgen receptor (AR) and LHR staining was performed in a single set of sections from the testes. All primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA) and employed at a dilution of 1:200. Immunohistochemistry was performed using methods described in (Krajnak, Miller, and Waugh 2018). Briefly, sections were fixed in 4% paraformaldehyde in 0.1 M PBS, rinsed, incubated in 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity, rinsed, and incubated in primary antibody diluted in 0.1 M PBS, 0.3% Triton-X 100 and 10% normal serum (goat or donkey; Santa Cruz Biotechnology) overnight at 4°C. The following day sections were rinsed, incubated in w Cy3-labeled second antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:800 in 0.1 M PBS, 0.3% Tx for 1 hr at RT, rinsed, air dried, and cover-slipped with Prolong-Gold with DAPI (Thermofisher). Sections were viewed using an Olympus BX63 fluorescent microscope and photomicrographs made at a magnification of 200 × using a DP73 camera and Cell Sense version 510 (Fisher Scientific, Indianapolis IN). The area labeled and intensity of the immunolabeling was quantified in each image using NIH Image. Average labeling is staining intensity for each animal was calculated by averaging the values from each animal. These mean values were used in the analyses described below.

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

qRT-PCR was performed to determine if exposure to WBV resulted in changes in transcript levels in the pituitary, ovaries, uteri, testes, and prostates of exposed animals using methods described in (Krajnak et al. 2010, 2009, 2007). RNA was isolated from each tissue using RNAeasy lipid Miniprep kits (cat # 74804; Qiagen, Valencia, CA), and first-strand cDNA was synthesized from 1 μg of total RNA using a Reverse Transcription System (Invitrogen; Carlsbad, CA). Melt curves were run for each transcript using each tissue. Samples that did not show a single defined melt peak in the 80°C range were not included in the dataset. To determine if the treatment resulted in a change in transcript levels, fold changes from the same-day controls were calculated. This was conducted by calculating the mean response for the control group and then subtracting the individual cycle time (CT) values for each sample from the average of the controls. Transcript levels for anti-oxidant enzymes, cytokines, factors involved in regulating cell cycle, and steroid hormone and anterior pituitary hormone receptors were measured in each tissue.

Analyses

Body weights were analyzed using a repeated-measures ANOVA for all pre-vibration exposure measures to determine if there were maintained effects. Post-hoc pairwise comparisons were made using Student's t-tests. The number of females displaying 4-day estrous cycles prior to the beginning of the exposure, and after 10 days of exposure, was analyzed using a Chi^2 analysis. To determine if there were acute effects of vibration on body weight and sensitivity to applied pressure, the difference between pre- and post-exposure measurements were calculated and analyzed using Student's t-tests. All biological data were also analyzed using Student's t-tests. Prior to performing the t-tests, data were examined to ensure that data for all measures were normally distributed. Differences with $p < .05$ were considered significantly different.

Results

Body weight

Mean body weights prior to vibration exposure were not significantly different in animals in the two conditions (females: restraint control; 230 ± 2.5 g, vibrated; 226 ± 1.93 g; males: restraint control 252.5 ± 3.65 g and vibrated 252.5 ± 6.34 g). Body weights between groups also were not markedly different over the course of the exposure (pre-exposure on days 1, 5, 10, and 11) in either females or males (Figure 2A and 2B, respectively). To determine if there were acute effects of WBV on body weights, post-exposure weights were also collected on days 5 and 10 of the experiment, and the post-pre exposure difference in weight was calculated and analyzed in both females and males (Figure 2C and 2D, respectively). All rats showed a reduction in body weight after exposure on day, however, the fall was greater in WBV-exposed than controls. On day 10 only males displayed a significant decrease in body weight (Figure 2D).

Estrous cycles

All females displayed normal 4-day estrous cycles prior to the beginning of the experiment. Over the course of the experiment, three rats (50%) exposed to vibration and one control rat (16.7%) stopped displaying normal 4-day estrous cycles. The decrease in the number of WBV-exposed animals exhibiting 4-day cycles pre- and post-exposure was significantly different. One rat from each treatment exhibited cornified epithelial cells in their vaginal smears for 3–4 days. The other two WBV-exposed animals did not display cytology consistent with any type of normal reproductive cycle (Figure 3).

Serum hormone concentrations

In females, circulating estradiol concentrations tended to be lower in vibration-exposed control animals, but this difference was not significant (Figure 4A). There were an insufficient number of animals at each stage of the cycle to determine if there was an interaction between exposure condition and day of the cycle. However, when estradiol concentrations were plotted using the day of the cycle as the independent variable, estradiol was higher on the day of proestrus (or on the day of ovulation) than on other days of the cycle (Figure 4B).

Circulating FSH levels in control and vibrated female rats were not markedly different (Figure 4C). When FSH concentrations were plotted over days of the cycle, these appeared to be lower on the day of estrous than on other days of the cycle (Figure 4D). There also were no significant differences between groups in circulating prolactin concentrations (Figure 4E).

Circulating testosterone levels were within the linear range of the assay in 1 out of 6 restraint control males and 3 out of 6 vibration-exposed animals (Figure 5A). There were no significant differences in circulating FSH concentrations in males (Figure 5B). However, circulating prolactin concentrations were significantly lower in males exposed to WBV than control animals (Figure 5C).

Ovarian function

The number of developing follicles were counted in H&E-stained sections (Figure 6A,B, G). Analyses revealed that females exposed to WBV had a lower number of developing follicles than control females. The reduction in developing follicle number was not accompanied by alterations in ERα immunostaining (Figure 6C, D, H), or ERβ staining (6 J), but accompanied by diminished LHR immunostaining (Figure 6E, F, I).

Testicular morphology

The mean number of spermatids was significantly decreased in males exposed to WBV (Figure 7A–7C). However, there were no marked differences in average number of seminiferous tubules, Leydig cells, or Sertoli cells (Figure 7D–7F, respectively).

Oxidative stress: Concentrations of reactive oxygen species (ROS) were measured in the pituitary, ovaries, testes, uteri, and prostates; (Table 1). Because one ovary was used for PCR and the other for histology, oxidative stress in the ovary was estimated by the intensity of nitrotyrosine immunostaining in sections. In all other tissues, ROS concentrations were measured using the fluorescent dye, 2′7’-dichlorofluorescien diacetate (DCFDA; (Krajnak et al. 2020)). In females, exposure to WBV did not markedly affect nitrotyrosine labeling in ovaries or fluorescence in the pituitary. However, exposure to WBV resulted in an increase in ROS (fluorescence) in uteri. In males, exposure to WBV did not alter ROS in the pituitary or testes but produced significant reduction in ROS-induced fluorescence in the prostate.

Transcript levels

WBV-induced changes in transcript levels in females are presented in Table 2. In the pituitary, there was a significant elevation in *Il6* expression in animals exposed to WBV, but no marked alteration in *Fshr*. In the ovary, there was a significant increase in *Per1* without marked change in *AchE* In the uterus, there were significant decreases in *Per1, Hif1, Ache* and *Pr*, and a significant rise in *Fshr*. No marked alterations were noted in *Il6* and Er_{α}.

Average changes in transcript levels in males exposed to control conditions or WBV are presented in Table 3. In the pituitary of males, WBV resulted in a significant reduction in *iNos* without marked alteration in *eNos*. In the prostate, exposure to WBV induced

significant decreases in *Il1β* and *Lhr* without effect on *Tnfα*. In the testes, there were significant elevations in *Lhr, AchE*, and *Bach* in response to WBV exposure.

Receptor expression

Western blots were used to measure the influence of WBV on ERα, AR, and LHR band density in the testes and prostate of males, and uteri of females (Figure 8). WBV exposure also did not markedly alter receptor expression in any of these tissues.

Discussion

The goal of the current study was to test the hypothesis that exposure to WBV induces changes in reproductive physiology that may affect fecundity and fertility. Male and female rats were exposed to WBV at the resonant frequency of the lumbar region of the torso (31.5 Hz). Ten days of exposure to WBV disrupted reproductive cycles in 50% of exposed females. Disruption of reproductive cycles may have been due to changes in circulating concentrations of gonadal (e.g. estradiol) and pituitary hormones, or alterations in the number or activity of hormone receptors (e.g. LHR). WBV also disrupted measures of reproductive function in males where exposure was associated with a reduction in circulating prolactin concentrations and elevation in circulating testosterone concentrations. WBV-exposed males also displayed a decrease in spermatid number. These results are consistent with epidemiological data suggesting that occupational exposure to WBV disrupts reproductive physiology in both males and females (Abdollahi et al. 2021; Haelterman et al. 2007; Skroder et al. 2020; Zarie et al. 2022).

In recent years, there has been an increase in the number of women working in occupations where they can be exposed to WBV (U.S. Bureau of Labor Statistics 2011, 2021). In fact, according to recent data reported by the U.S. Bureau of Labor Statistics, approximately 40% of bus drivers are women, and bus drivers are affected by WBV exposure (Golinko et al. 2020; Jonsson et al. 2015; U.S. Bureau of Labor Statistics 2011). Epidemiology investigators also suggested that exposure to occupational WBV may serve as a risk factor for miscarriage, preeclampsia, and adverse changes in fertility (Haelterman et al. 2007). Data in our study are consistent with the findings of epidemiological studies demonstrating that exposure to WBV resulted in a cessation of reproductive cycles in exposed females. In addition, exposure to WBV produced a reduction in the number of developing follicles, and LHR in the ovaries, along with a reduction in circulating estradiol concentrations. Exposure to WBV may induce these changes in reproductive function by acting through a number of different mechanisms.

Whole-body vibration WBV may affect reproductive cycles in females is it may stress animals, either physically by generating tissue stress and strain, or physiologically by altering blood flow, hormone synthesis, cell functioning or metabolism (Bailey and Silver 2014; Diez et al. 2020; Pioggiogalle, Jamshed, and Peterson 2018; Rosenwasser and Turek 2015; Yager and Chen 2007). In females, there was a reduction in the number of developing follicles. There was no marked change in FSH concentrations or FSH receptor in animals exposed to WBV suggesting that the hormonal cue that stimulates follicular development was not affected by WBV. Developing follicles synthesize estradiol which then acts on

the pituitary to further stimulate release of LH and FSH. Because there was a decrease in number of follicles, there was a fall in estradiol levels, and this may have led to a reduction in LH release and number of LHR in the ovaries. Unfortunately, it was not possible to measure LH concentrations in the current study as there was insufficient amount of serum to perform all the assays. It is also possible that WBV disrupted circadian rhythms because animals were exposed to vibration during the time they would normally be sleeping (Sciarra et al. 2020). However, this seems unlikely because control animals were also exposed at the same time and did not exhibit changes in the expression of normal reproductive cycles. Finally, it may be that the metabolic disruption produced by vibration exposure, or physical stress and strain on the ovaries induced changes in follicular development (Pioggiogalle, Jamshed, and Peterson 2018; Yager and Chen 2007). Additional studies need to be performed to determine the exact mechanisms.

Whole-body vibration also may have disrupted blood flow to the ovaries and uterus in exposed females and consequently altered the function of these organs. Previously Nakamura et al. (1995) examining the effects of WBV in pregnant rats demonstrated that blood flow to the uterus was significantly reduced in vibration-exposed animals. Although blood flow or vascular responsiveness in blood vessels supplying blood flow to the uterus or the ovary was not measured in the current study, alterations in transcript levels of factors that regulate blood flow in the ovary and uterus suggest that vibration may have induced vasodilation which might then enhance blood flow in animals exposed to WBV. For example, WBV exposure resulted in a reduction in *AchE* transcript expression in both the ovary and uterus. Acetylcholinesterase (AChE) is the primary enzyme that metabolizes acetylcholine (ACh) and regulates its activity at the receptor sites. ACh is one of the major neurotransmitters mediating vasodilation (Kennedy et al. 1999), and a reduction in metabolism of ACh enables the transmitter to act at receptors for an extended period and enhance vasodilation by stimulating release of nitric oxide (Deshwal et al. 2022; Kennedy et al. 1999; Kimura et al. 1985). WBV was also associated with diminished expression of *Hif1a* and *Il6* in uteri of females. *Hif1a* expression rose in response to tissue hypoxia and may affect vascular function, plasticity, and remodeling (Lee et al. 2004). Elevations in *Hif1a* are also often detected when there is a reduction in blood flow to tissue (i.e., diminished blood flow is usually associated with hypoxia (Lee et al. 2004). The findings that *Hif1a* and *Il6* expression were reduced in the uteri of WBV-exposed animals suggest that WBV exposure may have affected blood flow to this organ by mediating the expression of these factors and by affecting vasodilation and vascular remodeling (Deshwal et al. 2022; Krajnak et al. 2010).

In females, WBV increased measures of oxidative stress in the uterus. Although increases in oxidative stress may exert negative effects on cell functions, ROS might also stimulate vasodilation (Agarwal, Gupta, and Sharma 2005). Therefore, these observations are also consistent with the postulation that under the current exposure conditions, WBV may have initiated vasodilation rather than vasoconstriction. Although previously Nakamura et al. (1995) found that WBV reduces blood flow to the uterus, there are a number of factors that may account for differences in these findings. For example, in the study with pregnant rats, the frequency was lower (8 Hz) than it was in the current experiment, but the acceleration of exposure was higher (10 m/s^2) than it was in this experiment (0.3 g or 2.9 m/s^2). It is

possible that the lower acceleration used in the current study stimulated vasodilation rather than vasoconstriction (Bovenzi, Griffin, and Ruffel 1995; Bovenzi, Lindsell, and Griffin 1998), or that the duration of the exposure was not long enough to induce a reduction in blood flow. It is also possible that hormonal differences (Eid et al. 2007; Yager and Chen 2007) or differences in the size and vascularization of the uterus in pregnant and non-pregnant rats contributed to differing responses. Additional studies are needed to determine the mechanisms underlying WBV-induced effects on uterine function in animals that are and are not pregnant and may aid in understanding the mechanisms by which WBV induces preeclampsia and induces pre-term births.

Exposure to WBV may have also resulted in metabolic or general physiological stress in treated animals. In the current experiment, both male and female rats displayed significant reductions in pre-post exposure weights on days 5 and 10 of treatment. Although WBV-exposed animals regained weight after exposure, and pre-exposure weights never differed between control and exposed animals, stress induced by exposure, or by trying to maintain normal body weight and growth may have disrupted neuroendocrine function leading to cessation of normal reproductive cycles in females (Longo and Panda 2016; Mills and Kuohung 2019; Sciarra et al. 2020).

As mentioned above, maintenance of internal biological and physiological rhythms is also critical for normal reproductive functions in both males and females, and changes in feeding behavior in vibration-exposed animals may have altered expression of rhythms in reproductive tissue physiology. Food intake, along with the period of light and dark during the 24 hr cycle, serves as factors for entraining internal biological rhythms (Hosono et al. 2021). Internal rhythms are partially generated by a number of “clock” genes, one of which is the *Period1* or *Per1* gene (Rosenwasser and Turek 2015). The change in *Per1* expression in the uterus and ovary may have been attributed to a shift in circadian expression of these genes rather than an overall decrease in expression (Fahrenkrug et al. 2006; He et al. 2007). A shift or general change in the expression of this gene in these tissues may disrupt cyclic development of follicles and generation of reproductive cycles (Fahrenkrug et al. 2006), and potentially cyclic alterations in the uterus that are necessary to support implantation and maintenance of pregnancy. Additional studies examining changes in expression of the transcription of *Per1*, other clock genes, and hormones and receptors involved in regulating reproduction, over a 24 hr period after treatment would determine if exposure to WBV disrupts the circadian clock in these tissues and if these observed alterations in part underlie reproductive dysfunction.

In males, treatment with WBV reduced spermatid numbers and circulating prolactin concentrations, but increased levels of circulating testosterone. The change in circulating prolactin concentrations may have been related to stress as evidenced by alterations in eating patterns and metabolic stress which were found to alter circulating prolactin concentrations and testosterone concentrations (Corona et al. 2009; Koivisto et al. 2009; Krajnak et al. 1994; Seuscun et al. 1985). Both elevations and decreases in circulating prolactin concentrations have been associated alterations in reproductive function in males (Krajnak et al. 1995; Koivisto et al. 2009; Seuscun et al. 1985). However, as stated above, additional work needs to be performed to determine if the changes in prolactin and testosterone were

the result of a general change in circulating hormone concentrations or due to a shift in rhythmic release of these hormones. WBV exposure also resulted in diminished levels of *eNos* and *iNos* in the pituitary. If the fall in transcript expression was accompanied by a reduction in enzyme concentrations, these changes may have resulted in diminished blood flow within or to the pituitary potentially affecting gonadotropin and prolactin synthesis, release, and delivery to peripheral tissues.

There was also a significant rise in the expression of the *Bach1* gene in the testes of males exposed to WBV. This gene plays a role in regulating the cell cycle, oxidative stress, and immune responses. Because cell cycle regulation and oxidative stress play a role in regulating both steroid hormone synthesis and rate of sperm cell maturation, alterations in the expression of this gene may contribute to the change in number of spermatids (Agarwal et al. 2014; Dutta et al. 2021; Zhang et al. 2018).

Previous investigators found that workers exposed to WBV exhibit an increased risk of developing prostatic cancer (Nadalin et al. 2012; Young et al. 2009). In the current study, exposure to WBV resulted in a reduction in oxidative stress, increased expression of the cytokines *Tnfα* and *Il1β*, and expression of *Lhr*. An imbalance in the expression of these factors, or in levels of ROS was associated with an enhanced risk of prostate cancer. However, most studies demonstrated that over-expression rather than a reduced expression contributed to dysfunction (Cao et al. 2009; Crawford and Schally 2020; Kiss and Ghosh 2016; Stroomberg et al. 2020). Because this study only looked at a certain time point, the changes observed in expression of these genes may be due to a shift in expression of the circadian rhythm (Cao et al. 2009). Because changes in reproductive function in response to WBV may be frequency-dependent examining the effects of exposure to WBV at 20 Hz (closer to the resonant frequency of the lumbar region in males) may provide more information as to how WBV affects neuroendocrine function in males, and the mechanisms that might underlie that change. For example, previously, Krajnak et al. (2010); Krajnak et al. (2012a); 2012b) demonstrated that disruption of normal physiological function and injury are increased at the resonant frequency. Therefore, the effects of WBV exposure on expression of certain genes, circulating hormone concentrations and receptor levels may have varied had the treatment been closer to the resonant frequency of the torso in males.

Conclusions

Our observations are consistent with the findings of epidemiological studies (Skroder et al. 2020, 2021) and the hypothesis that exposure to WBV affects reproductive function in both females and males. Data from the current study also suggest that WBV may disrupt reproductive function by (1) affecting hormone synthesis and release by the pituitary and gonads, (2) blood flow to the reproductive organs, and (3) possibly by inducing an imbalance of ROS levels, inflammatory factors, and factors involved in mediating circadian function within the reproductive system. Additional studies examining exposures to the resonant frequency of the torso of males and measuring additional time points after exposure in males and females may provide more information regarding the mechanisms underlying development of reproductive function in workers exposed to WBV.

Acknowledgments

This work was funded by the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

Funding

This work was supported by the This work was funded by NIOSH as part of the official duties of the authors [NIOSH 9390BMW].

Data Access Statement

The data used to generate table and figures in this paper, along with a brief review of the paper can be found at https://www.cdc.gov/niosh/data/default.html.

References

Abdollahi MB, Dehghan SF, Balochkhaneh FA, Moghadam MA, and Mohammadi H. 2021. Comparison of mice sperm parameters exposed to some hazardous physical agents. Environ Anal Health Toxicol 36 (3):e2021013. doi:10.5620/eaht.2021013. [PubMed: 34353003]

Agarwal A, Gupta S, and Sharma RK. 2005. Role of oxidative stress in female reproduction. Reprod. Biol. Endocrinol. 3 (1):1–22. doi:10.1186/1477-7827-3-28. [PubMed: 15642115]

Agarwal A, Virk G, Ong C, and duPlessis S. 2014. Effect of oxidative stress on male reproduction. World J Mens Health 32 (1):1–17. doi:10.5534/wjmh.2014.32.1.1. [PubMed: 24872947]

Bailey M, and Silver R. 2014. Sex differences in circadian timing systems: Implications for disease. Front Neuroendocrinol 35 (1):111–39. doi:10.1016/j.yfrne.2013.11.003. [PubMed: 24287074]

Bovenzi M 2002. Finger systolic blood pressure indices for the diagnosis of vibration-induced white finger. Int Arch Occup Environ Health 75 (1):20–28. doi:10.1007/s004200100274. [PubMed: 11898873]

Bovenzi M, Griffin MJ, and Ruffel CM. 1995. Acute effects of vibration on digital circulatory function in healthy men. Occup Environ Med 52 (12):111–39. doi:10.1136/oem.52.12.834.

Bovenzi M, Lindsell CJ, and Griffin MJ. 1998. Duration of acute exposures to vibration and finger circulation. Scand. J. Work Environ. Health 24 (2):20–28. doi:10.5271/sjweh.290.

Cao Q, Gery S, Dashti A, Yin D, Zhou Y, Gu J, and Koeffler HP. 2009. A role for the clock gene *Per1* in prostate cancer. Endocrinology 69:7619–25.

Charles LE, Ma CC, Burchfiel CM, and Dong RG. 2018. Vibration and ergonomic exposures associated with musculoskeletal disorders of the shoulder and neck. Saf Health Work 9 (2):125–32. doi:10.1016/j.shaw.2017.10.003. [PubMed: 29928524]

Cora MC, Kooistra L, and Travlos G. 2015. Vaginal cytology of the laboratory rat and mouse: Review and criteria for the staging of the estrous cycle using stained vaginal smears. Toxicol Pathol 43 (6):776–93. doi:10.1177/0192623315570339. [PubMed: 25739587]

Corona G, Mannucci E, HJannini EA, Lotti F, Balercia G, Forti G, and Maggi M. 2009. Hypoprolactinemia: A new clinical syndrome in patients with sexual dysfunction. Endocrinology 6:1457–66.

Crawford ED, and Schally AV. 2020. The role of FSH and LH in prostate cancer and cardiometabolic co-morbidities. Can J Urol 27 (2):10167–73. [PubMed: 32333736]

Croteau A, Marcoux S, and Brisson C. 2007. Work activity in pregnancy, preventive measures, and the risk of preterm delivery. Am. J. Epidemiol. 166 (8):951–65. doi:10.1093/aje/kwm171. [PubMed: 17652310]

Deshwal A, Gill AK, Nain S, Patra D, and Maiti S. 2022. Inhibitory effect of nucleotides on acetylcholine esterase activity and its microflow-based actuation in blood plasma. Chem. Commun. (Camb.) 58 (21):3501–04. doi:10.1039/D2CC00029F. [PubMed: 35194626]

Diez JJ, Plano SA, Caldart C, Bellone G, Simonelli G, Brangold M, Cardinali DP, Golombek D, Pérez Chada D, and Vigo DE. 2020. Sleep misalignment and circadian rhythm impairment in

long-haul bus drivers under a two-up operations system. Sleep Health 6 (3):374–86. doi:10.1016/j.sleh.2019.12.011. [PubMed: 32081596]

Dong RG, Welcome DE, Xu XS, Warren C, McDowell TW, Wu JZ, and Rakheja S. 2012. Mechanical impedances distributed at the fingers and palm of the human hand in three orthogonal directions. J. Sound Vib. 331 (5):1191–206. doi:10.1016/j.jsv.2011.10.015.

Du BB, Bigelow RP, Wells HW, Hall DP, Johnson PW, and Johnson PW. 2018. The impact of different seats and whole-body vibration exposures on truck driver vigilance and discomfort. Ergonomics 61 (4):528–37. doi:10.1080/00140139.2017.1372638. [PubMed: 28845747]

Dutta S, Sengupta P, Slama P, and Roychoudhury S. 2021. Oxidative stress, testicular inflammatory pathways and male reproduction. Mol Sci 22 (18):10043. 10.3390/ijms221810043.

Eid AH, Maiti K, Mitra S, Chotani MA, Flavahan S, Bailey SR, Thompson-Torgerson CS, and Flavahan NA. 2007. Estrogen increases smooth muscle expression of α 2C - adrenoceptors and cold-induced constriction of cutaneous arteries. Am. J. Physiol. Heart Circ. Physiol. 293 (3):H1955–H1961. doi:10.1152/ajpheart.00306.2007. [PubMed: 17644575]

Eng A, Mannetje AT, McLean D, Ellison-Loschmann L, Cheng S, and Pearce N. 2011. Gender differences in occupational exposure patterns. Occup Environ Med 68 (12):888–94. doi:10.1136/oem.2010.064097. [PubMed: 21486991]

Fahrenkrug J, Georg B, Hannibal J, Hindersson P, and Grasm S. 2006. Diurnal rhymicity of the clock genes *Per1* and *Per2* in the rat ovary. Encocrinology 147 (8):528–37. doi:10.1210/en.2006-0305.

Ferrell MC, Giza RJ, and Shibao CA. 2020. Race and sex differences in cardiovascular autonomic regulation. Clin. Auton. Res. 30 (5):371–79. doi:10.1007/s10286-020-00723-z. [PubMed: 32894376]

Golinko V, Cherberyachko S, Deryugin O, Tretyak O, and Dusmatova O. 2020. Assessment of the risks of occupational diseases of the passenger bus drivers. Saf Health Work 11 (4):543–49. doi:10.1016/j.shaw.2020.07.005. [PubMed: 33329922]

Govers ME, Nolan AJ, Hassan M, and Oliver ML. 2021. Relationships between height, mass, body mass index and truck muscle activation during seated whole-body vibration exposure. Vibration 4 (4):822–35. doi:10.3390/vibration4040046.

Griffin M, Bovenzi M, and Nelson CM. 2003. Dose-response patterns for vibration-induced white finger. Occup Environ Med 60 (1):16–26. doi:10.1136/oem.60.1.16. [PubMed: 12499452]

Haelterman E, Marcoux S, Croteau A, and Dramaix M. 2007. Population-based study on occupational risk factors for preeclampsia and gestational hypertension. Scand J Work Environ Health 33 (4):304–17. doi:10.5271/sjweh.1147. [PubMed: 17717623]

Hartikainen AL, Sorri M, Anttonen H, Tuimala R, and Laara E. 1994. Effect of occupational noise on the course and outcome of pregnancy. Scand J Work Environ Health 20 (6):444–50. doi:10.5271/sjweh.1376. [PubMed: 7701290]

He PJ, Hirata M, Yamauchi N, and Hattori M-A. 2007. Up-regulation of Per1 expression by estradiol and progesterone in the rat uterus. J. Endocrinol. 194 (3):511–19. doi:10.1677/JOE-07-0172. [PubMed: 17761890]

Hosono T, Ono M, Daikoku T, Mieda M, Nomura S, Kagami K, Iizuka T, Nakata R, Fujiwara T, Fujiwara H, et al. 2021. Time-restricted feeding regulates circadian rhythm of murine uterine clock. Curr Dev Nutr 5 (5):nzab064. doi:10.1093/cdn/nzab064. [PubMed: 33981944]

Jeyaraj SC, Unger NT, Eid AH, Mitra S, El-Dahdah P, Quilliam LA, Flavahan NA, and Chotani MA. 2012. Cyclic AMP-Rap1A signaling activates RhoA to induce α 2c - adrenoceptor translocation to the cell surface of microvascular smooth muscle cells. Am. J. Physiol., Cell Physiol. 303 (5):C499–C511. doi:10.1152/ajpcell.00461.2011. [PubMed: 22621783]

Jonsson PM, Rynell PW, Hagberg M, and Johnson PW. 2015. Comparison of whole-body vibration exposures in buses: Effects and interactions of bus and seat design. Ergonomics 58 (7):1133–42. doi:10.1080/00140139.2014.961568. [PubMed: 25290555]

Jungwirth A, Galvan J, Halmos PG, Cai R-Z, Cai R-Z, Groot K, Schally AV, and Schally AV. 1997. Luteinizing hormone-releaseing hormone antagonist cetrorelix (SB-75) and bombesin antagonist RC-3940-II inhibit the growth of androgen-independent PC-3 prostate cancer in nude mice. The Prostate 32 (3):164–72. doi:10.1002/(SICI)1097-0045(19970801).AID-PROS2>3.0.CO;2-L. [PubMed: 9254895]

Kennedy G, Khan F, McLaren M, and Belch JJ. 1999. Endothelial activation and response in patients with hand arm vibration syndrome. Eur. J. Clin. Invest. 29 (7):577–81. doi:10.1046/j.1365-2362.1999.00502.x. [PubMed: 10411662]

Kimura K, Low DA, Keller DM, Davis SL, and Crandall CG. 1985. Cutaneous blood flow and sweat rate responses to exogenous administration of acetylcholine and methacholine. J. Appl. Physiol. 102 (5):1856–61. doi:10.1152/japplphysiol.01069.2006.

Kiss Z, and Ghosh PM. 2016. Circadian rhythmicity and the influence of “clock” genes on prostate cancer. Endocr. Relat. Cancer 23 (11):T123–T134. doi:10.1530/ERC-16-0366. [PubMed: 27660402]

Koch L, Gaese BH, and Nowatny M. 2022. Strain comparison in rats differentiates strain-specific from more general correlates of noise-induced hearing loss and tinnitus. J. Assoc. Res. Otolaryngol 23 (1):59–73. doi:10.1007/s10162-021-00822-2. [PubMed: 34796410]

Koivisto MB, Eschricht F, Urhausen C, Hoppen HO, Beyerbach M, Oei C, and Gunzel-Apel AR. 2009. Effects of short-term hyper- and hypoprolactineaemia on hormones of the pituitary gonad and -thyroid axis on seme quality in male beagles. Reprod. Domest. Anim 44:320–25. doi:10.1111/j.1439-0531.2009.01425.x.

Krajnak K, Kan H, Russ KA, McKinney W, Waugh S, Zheng W, Kashon ML, Johnson C, Cumpston J, and Fedan JS. 2020. Biological effexts of inhaled hydraulic fracturing sand dust. VI. Cardiovascular effects. Toxicol. Appl. Pharmacol. 406:1115242. doi:10.1016/j.taap.2020.115242.

Krajnak K, Lookingland KJ, and Nunez AA. 1995. Seasonal changes in median eminence dopamine in male syrian hamsters: Role of the gonads and duration of exposure to short days. Brain Res. Bull. 37 (6):617–22. doi:10.1016/0361-9230(95)00053-H. [PubMed: 7670886]

Krajnak K, Manzanares J, Lookingland KJ, and Nunez AA. 1994. Gender differences in tuberoinfundibular dopaminergic neuronal activity in a photoperiodic rodent (*Mesocricetus auratus*). Brain Res. 634 (1):159–62. doi:10.1016/0006-8993(94)90270-4. [PubMed: 8156387]

Krajnak K, Miller GR, Miller GR, Johnson C, Kashon ML, and Kashon ML. 2010. Vascular responses to vibration are frequency dependent. Journal of Occupational & Environmental Medicine 52 (6):584–94. doi:10.1097/JOM.0b013e3181e12b1f. [PubMed: 20523237]

Krajnak K, Miller GR, and Waugh S. 2018. Contact area affects frequency-dependent responses to vibration in the peripheral vascular and sensorineural systems. J. Toxicol. Environ. Health Part A 81 (1–3):6–19. doi:10.1080/15287394.2017.1401022.

Krajnak K, Miller GR, Waugh S, Johnson C, and Kashon ML. 2012b. Characterization of frequency-dependent responses of the sensorineural system to repetitive vibration. J. Occup. Environ. Med. 54 (8):1010–16. doi:10.1097/JOM.0b013e318255ba74. [PubMed: 22785326]

Krajnak K, Riley DA, Wu J, McDowell T, Welcome DE, Xu XS, and Dong RG. 2012a. Frequency-dependent effects of vibration on physiological systems: Experiments with animals and other human surrogates. Ind Health 50 (5):343–53. doi:10.2486/indhealth.MS1378. [PubMed: 23060248]

Krajnak K, Rosewell KL, and Wise PM. 2001. Fos-induction in gonadotropin-releasing hormone neurons receiving vasoactive intestinal polypeptide innervation is reduced in middle-aged female rats. Biol. Reprod. 64 (4):1160–64. doi:10.1095/biolreprod64.4.1160. [PubMed: 11259263]

Krajnak K, Waugh S, Johnson C, Miller R, and Kiedrowski M. 2009. Vibration disrupts vascular function in a model of metabolic syndrome. Ind Health 47 (5):533–42. doi:10.2486/indhealth.47.533. [PubMed: 19834263]

Krajnak K, Waugh S, Miller R, Baker B, Geronilla K, Alway SE, and Cutlip RG. 2006. Proapoptotic factor BAX is increased in satellite cells in the tibialis anterio muscle of old rats. Muscle Nerve 34 (6):720–30. doi:10.1002/mus.20656. [PubMed: 16967487]

Krajnak K, Waugh S, Wirth O, and Kashon ML. 2007. Acute vibration reduced Aβ nerve fiber sensitivity and alters gene expression in the ventral tail nerves of rats. Muscle Nerve 36 (2):197–205. doi:10.1002/mus.20804. [PubMed: 17541999]

Lee J-W, Bae S-H, Jeong J-W, Kim S-H, and Kim K-W. 2004. Hypoxia-inducible factor (HIF-1)alpha: Its protein stability and biological functions. Exp. Mol. Med. 36 (1):1–12. doi:10.1038/emm.2004.1. [PubMed: 15031665]

Liou JT, Lui PW, Lo YL, Liou L, Wang SS, Yuan HB, Chan KH, and Lee TY. 1999. Normative data of quantitative thermal and vibratory thresholds in normal subjects in Taiwan: Gender and age effect. Zhonghua Yi Xue Za Zhi (Taipei) 62 (7):431–37. [PubMed: 10418177]

Longo VD, and Panda S. 2016. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. Cell Metab. 14 (6):1048–59. doi:10.1016/j.cmet.2016.06.001.

Milanese C, Piscitelli F, Simoni C, Pugliarello R, and Zancanaro C. 2012. Effects of whole-body vibration with or without localized radiofrequency on anthropometry, body composition, and motor performance in young non-obese women. J Alter Complement Med 18 (1):69–75. doi:10.1089/acm.2010.0324.

Mills J, and Kuohung W. 2019. Impact of circadian rhythms on female reproduction and infertility treatment success. Curr Opin Endocrinol Diabetes Obes 26 (6):317–21. doi:10.1097/MED.0000000000000511. [PubMed: 31644470]

Miyashita K, Morioka I, Tanabe T, Iwata H, and Takeda S. 1992. Symptoms of construction workers exposed to whole body vibration and local vibration. Int Arch Occup Environ Health 64 (5):347–51. doi:10.1007/BF00379545. [PubMed: 1487332]

Morgan LJ, and Mansfield NJ. 2014. A survey of expert opinion on the effects of occupational exposures to trunk rotation and whole-body vibration. Ergonomics 57 (4):563–74. doi:10.1080/00140139.2014.887785. [PubMed: 24697831]

Nadalin V, Kreiger N, Parent ME, Salmoni A, Sass-Kortsak A, Siemiatycki J, Sloan M, and Purdham J. 2012. Prostate cancer and occupational whole-body vibration exposure. Ann. Occup. Hyg. 56 (8):968–74. doi:10.1093/annhyg/mes010. [PubMed: 22539558]

Nakamura H, Ohsu W, Nagase H, Okazawa T, Yoshida M, and Okada A. 1995. Uterine circulatory dysfunction induced by whole-body vibration and its endocrine pathogenesis in the pregnant rat. Eur. J. Appl. Physiol. 72 (4):292–96. doi:10.1007/BF00599687.

Pacurari M, Waugh S, and Krajnak K. 2019. Acute vibration induces peripheral nerve sensitization in a rat tail model: Possible role of oxidative stress and inflammation. Neuroscience 398:263–72. doi:10.1016/j.neuroscience.2018.12.010. [PubMed: 30553794]

Pioggiogalle E, Jamshed H, and Peterson CM. 2018. Circadian regulation of glucose, lipid, and energy metabolism in humans. Metabolism 84:11–27. doi:10.1016/j.metabol.2017.11.017. [PubMed: 29195759]

Pukkala E, Martinsen JI, Lynge E, Gunnarsdottir HK, Sparen P, Tryggvadotttir L, Weiderpass E, and Kjaerheim K. 2009. Occupation and cancer-follow-up of 15 million people in five nordic countries. Acta Oncol 48 (5):646–790. doi:10.1080/02841860902913546. [PubMed: 19925375]

Rosenwasser AM, and Turek FW. 2015. Neurobiology of circadian rhythm regulation. Sleep Med Clin 10 (4):403–12. doi:10.1016/j.jsmc.2015.08.003. [PubMed: 26568118]

Rushton L, Bagga S, Bevan R, Brown TP, Cherrie JW, Holmes P, Fortunato L, Slack R, Van Tongeren M, Young C, et al. 2010. Occupation and cancer in Britian. Br. J. Cancer 102 (9):1428–37. doi:10.1038/sj.bjc.6605637. [PubMed: 20424618]

Sciarra F, Franceschini E, Campolo F, Gianfrilli D, Pallotti F, Paol ID, Isidori AM, and Venneri MA. 2020. Disruption of circadian rhythms: A crucial factor in the etiology of infertility. Int J Mol Sci 21 (11):3943. doi:10.3390/ijms21113943. [PubMed: 32486326]

Scott A, and Stout M 2013. Musculoskeletal disorder risk assessment tool for train drivers. Rail Human Factors: Supporting Reliability, Safety and Cost Reduction: Dadashi N and Scott JRA Eds. Wilson, A. Mills CRC Press. pp. 101–08.

Seuscun MO, Scorticati C, Chiauzzi VA, Chemes HE, Rivarola MA, and Calandra RS. 1985. Induced hypoprolactinemia and testicular steroidogenesis in man. J. Androl. 6 (1):10–14. doi:10.1002/j.1939-4640.1985.tb00811.x. [PubMed: 3972716]

Skroder H, Pettersson H, Albin M, Gustavsson P, Rylander L, Norlen F, and Selander J. 2020. Occupational exposure to whole-body vibrations and pregnancy complications: A nationwide cohort study in Sweden. Workplace 77:691–98.

Skroder H, Pettersson H, Norlen F, Gustavsson P, Rylander L, Albin M, and Selander J. 2021. Occupational exposure to whole body vibrations and birth outcomes-A nationwide cohort study of Swedish women. Sci. Total Environ. 751:141476. doi:10.1016/j.scitotenv.2020.141476. [PubMed: 32871309]

Stoynev AG, and Minkova NK. 1997. Circadian rhythms of arterial pressure, heart rate and oral temperature in truck drivers. Occup Med 47 (3):151–54. doi:10.1093/occmed/47.3.151.

Stroomberg HV, Jorgenson A, Brasso K, Nielsen JE, Juul A, Frederiksen H, Jensen MB, and Roder MA. 2020. Novel functions of the luteinizing hormone/chroic gonadotropin receptor in prostate cancer cells and patients. PLoS One 15 (9):e0238814. doi:10.1371/journal.pone.0238814. [PubMed: 32881970]

U.S. Bureau of Labor Statistics. 2011. Women at Work. cited March 2011.

U.S. Bureau of Labor Statistics. 2021. Women in the labor force: a databook.

Wang WP, Bazrgari B, Shirazi-Adl A, Rakheja S, and Boileau PE. 2010. Biodynamic response and spinal load estimation of seated body in vibration using finite element model. Ind Health 48:557–64. doi:10.2486/indhealth.MSWBVI-34. [PubMed: 20953073]

Wang YC, Wang CC, Yao Y-H, and Wu W-T. 2021. Identification of a high-risk group of new-onset cardiovascular disease in occupational drivers by analyzing heart rat variability. Int J Environ Res and Public Health 18. doi:10.3390/ijerph182111486.

Waugh S, Kashon ML, Li S, Miller GR, Johnson C, and Krajnak K. 2016. Transcriptional pathways altered in response to vibration in a model of hand-arm vibration syndrome. J. Occup. Environ. Med. 58 (4):344–50. doi:10.1097/JOM.0000000000000705. [PubMed: 27058473]

Yager JD, and Chen JQ. 2007. Mitochondrial estrogen receptors—new insights into specific functions. Trends Endocrinol. Metab 18 (3):89–91. doi:10.1016/j.tem.2007.02.006. [PubMed: 17324583]

Young E, Kreiger N, Purdham J, and Sass-Kortsak A. 2009. Prostate cancer and driving occupations: Could whole body vibration play a role? Int Arch Occup Environ Health 82 (5):551–56. doi:10.1007/s00420-009-0403-z. [PubMed: 19242718]

Zarie S, Dehghan SF, Vaziri MH, Gilani MAS, and A. S. k. 2022. Assessment of semen quality of taxi drivers exposed to whole body vibration. J. Occup. Med. Toxicol 17 (1). doi:10.1186/s12995-022-00357-z.

Zhang X, Guo J, Wei X, Niu C, Jia M, Li Q, and Meng D. 2018. Bach1: function, regulation, and involvement in disease. Oxid. Med. Cell 2018:1347969. 10.1155/2018/1347969.

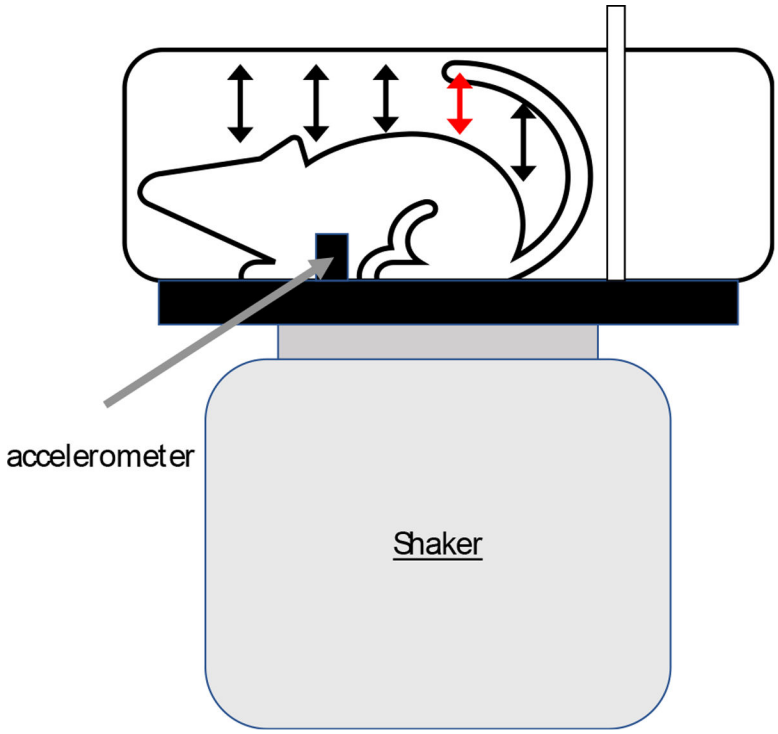

Figure 1.

This diagram depicts the exposure system used in the experiment. The black and red arrows above the animal show where measurements were taken using a laser vibrometer to determine the response to vibration at different frequencies. For this study, the resonant frequency collected near the lumbar spine (red arrow) was used for exposures. The gray arrow points to the accelerometer that was secured to the platform and measured the response of the platform during exposure to ensure the vibration input remained constant over the 4 hr exposure.

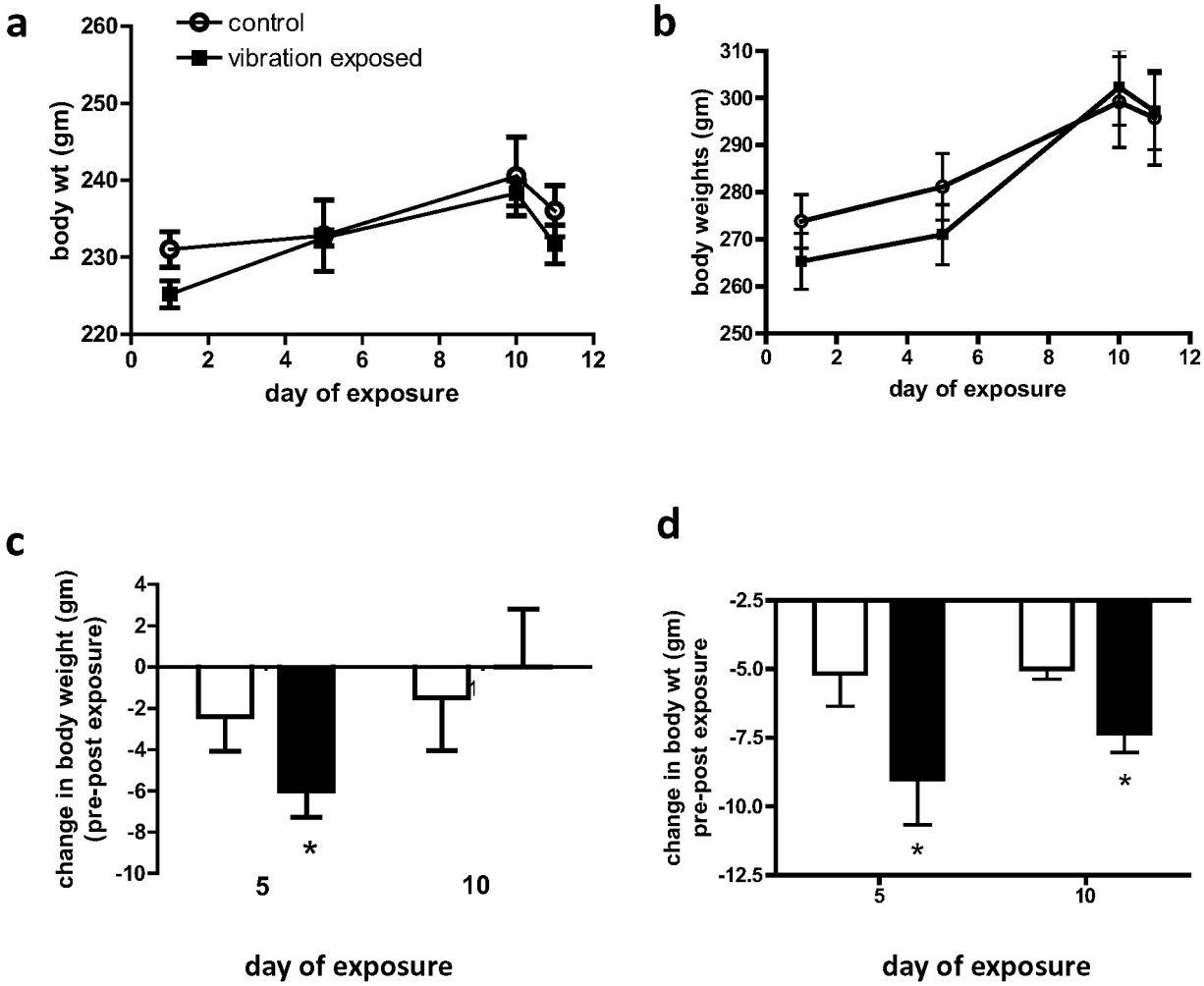

Figure 2.

Body weights (bw) of female (A) and male (B) rats over a 10-day exposure to WBV (mean ± sem in g). Both control and WBV exposed females and males gained weight as expected. WBV did not affect overall growth or weight gain. When body weights collected immediately following exposure (post) were compared to those collected immediately pre-exposure, both females (C) and males (D) displayed reductions in body weight (mean change in bw in gm pre-post exposure) as compared to same day controls on day 5 of the exposure (*$p < .05$). On day 10, only males displayed a significant reduction in body in body weight.

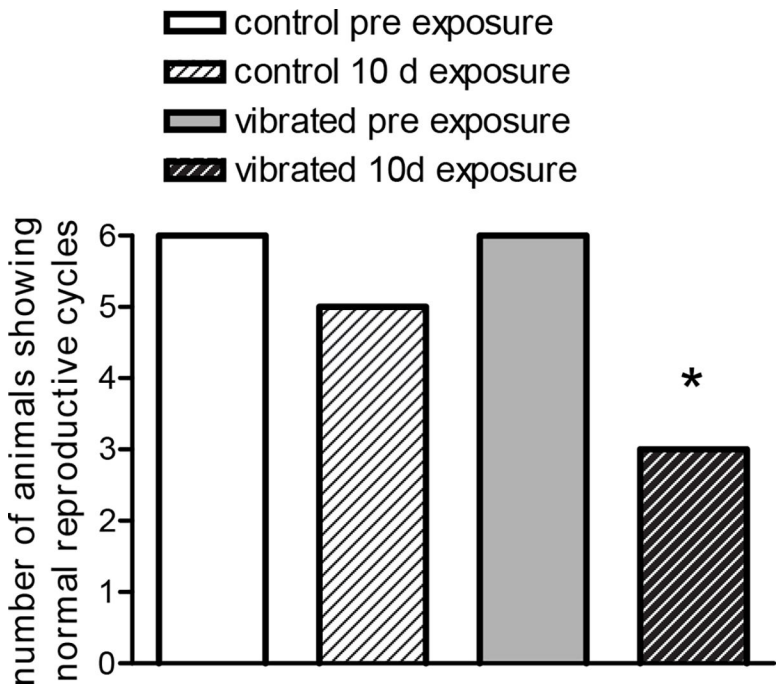

Figure 3.

The number of control and WBV exposed animals displaying normal 4 day estrous cycles after 10 days of exposure to restraint control or WBV (*$Chi^2 < 0.05$).

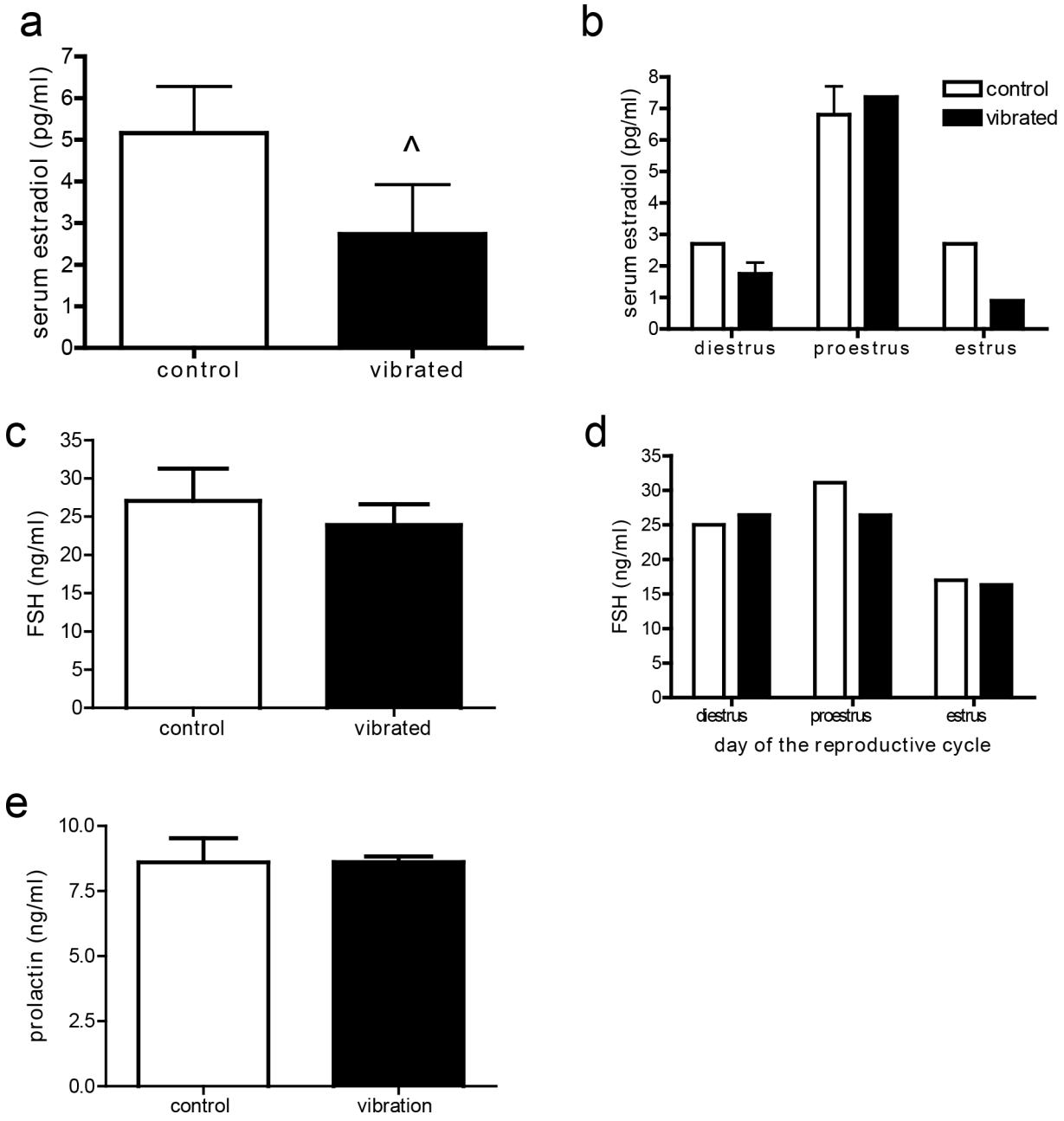

Figure 4.

Circulating hormone concentrations in females after 10 day exposure to control or WBV conditions. Circulating estradiol concentrations (A) were lower in WBV than control exposed females REMOVE. However, circulating estradiol concentrations were increased in females on the day of proestrus (the day of ovulation) in both groups of animals (B). Exposure to WBV did not alter circulating concentrations of FSH (C and D) or prolactin (E).

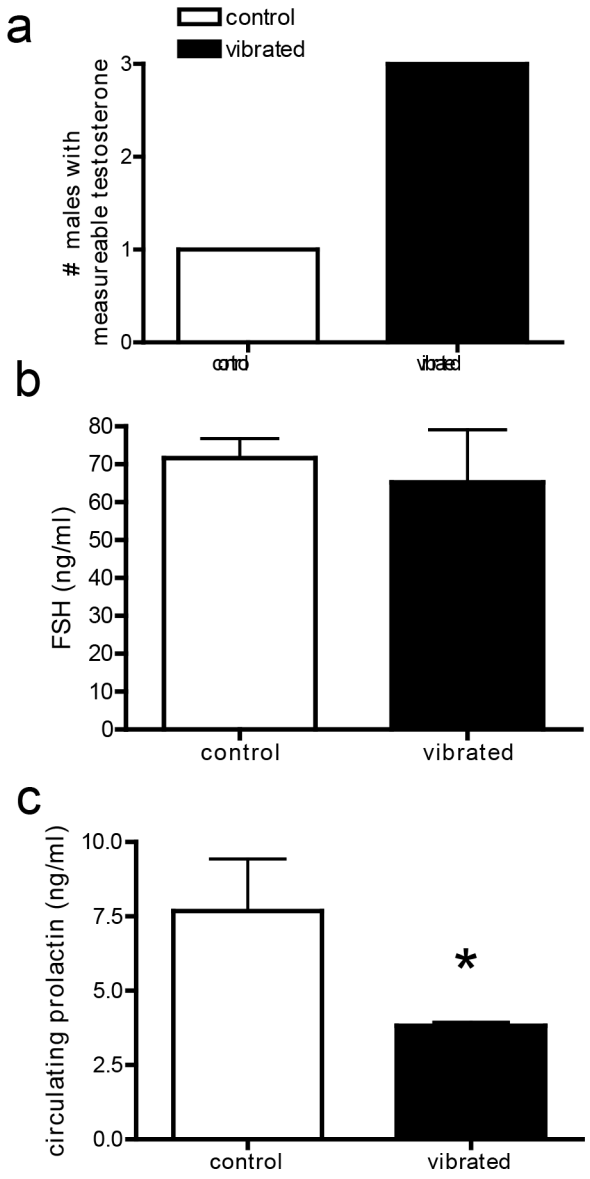

Figure 5.

Circulating hormone concentrations in males after 10 day exposure to control or WBV conditions. There were more males in the WBV group that displayed circulating testosterone concentrations that were within the measurable range than control males that had measurable testosterone concentrations (A). There were no differences in circulating FSH concentrations (B), and males exposed to WBV had significantly lower circulating concentrations of prolactin than control males (C; *p < .05).

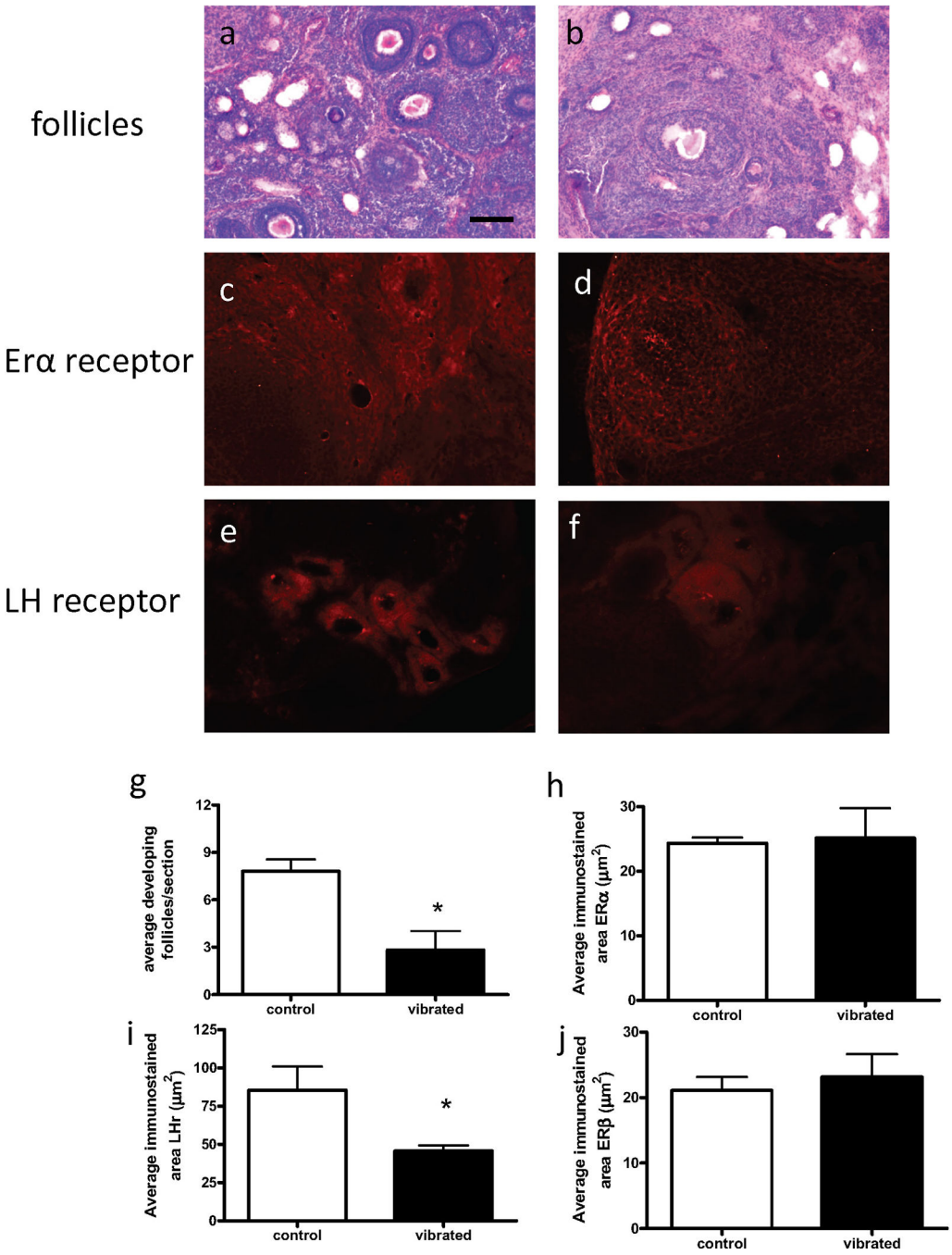

Figure 6.

The photomicrographs show H&E staining and immunostaining for Erα and LHr in ovaries from control (A, C, E) and WBV-exposed (B, D, F) animals. Quantification of developing ova in H&E sections showed that WBV resulted in a significant decrease in the number of developing ova (G), WBV exposure did not alter staining intensity for Erα or β (H and J) in the ovary. However, LHr levels were significantly lower in the ovaries of WBV than in control animals (I; *$p < .05$). Bar = 20 μm.

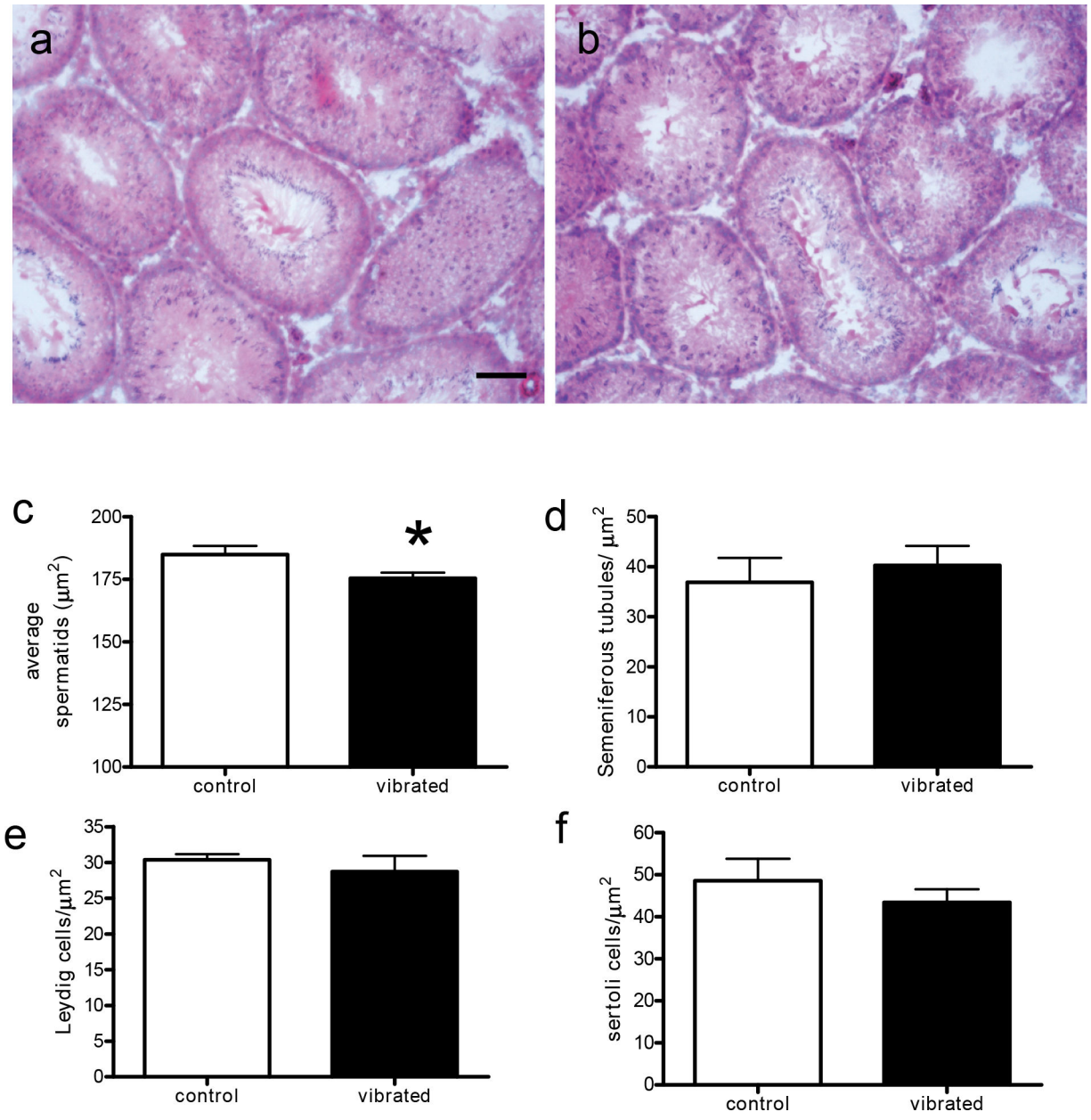

Figure 7.

Photomicrographs of H&E-stained testicular tissue from control (A) and WBV-exposed (B) males. The average number of spermatids (C) was reduced in males exposed to WBV. There were no significant differences in the number of seminiferous tubules (D), Leydig cells (E) or Sertoli cells (F) between control and WBV exposed animals (*p < .05). Bar = 20 µm.

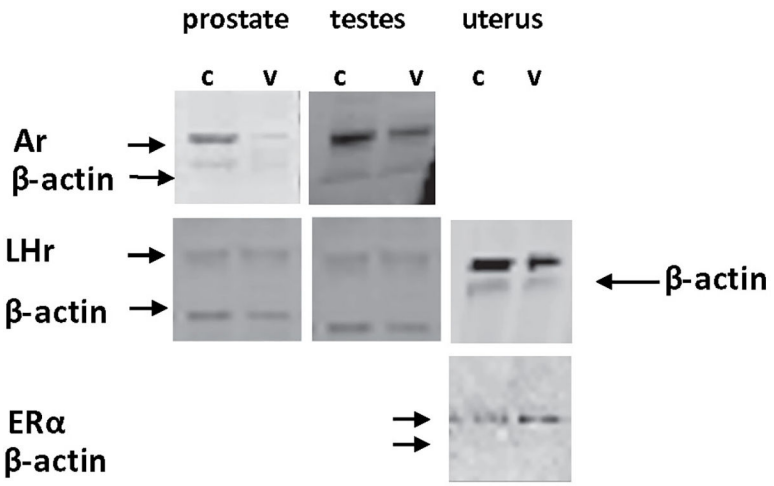

Figure 8.

Western analyses for Androgen receptor (Ar), luteinizing hormone receptor (LHr), and estrogen receptor α (Erα) in the prostate, testes, and uterus of male or female rats exposed to WBV. The bands on the blots represent bands from a control (C) and WBV-exposed (V; vibrated) animals. B-actin was used as a loading control and was not significantly different between groups. Exposure to WBV did not affect the expression of any of the receptors in these tissues (band densities in table).

Table 1.

Oxidative stress in reproductive tissues.

	Male		Female	
Tissue	**Control**	**Vibrated**	**Control**	**Vibrated**
pituitary	1.36 (0.39)	1.35 (0.40)	3.98 (0.42)	3.73 (0.52)
ovaries			7.92 (1.19)	9.85 (1.07)
uterus			**0.05 (0.02)**	**0.09 (0.02)** *
prostate	**159.96 (50.06)**	**46.26 (16.79)** *		
testes	840.94 (518.09)	477.10 (242.03)		

Measures of oxidative stress in the ovaries were estimated by measuring levels of nitrotyrosine immunostaining. However, for all other tissues, ROS levels measure represent the level of fluorescence/μg protein). Levels are mean levels of staining or fluorescence (± sem).

* Significant at $p < 0.05$ from control.

Table 2.

Fold changes in transcript expression in the pituitary, ovary and uterus of females exposed to control or WBV conditions.

	Pituitary		Ovary		Uterus	
	Control	**wbv**	**Control**	**wbv**	**Control**	**wbv**
Il1β	1.12 (0.24)	1.22 (0.06)	1.06 (0.15)	1.22 (0.21)	1.04 (0.12)	1.29 (0.15)
Il6	1.21 (0.43)	**3.28 (0.42)****	0.83 (0.05)	0.77 (0.08)	**1.17 (0.27)**	**0.68 (0.12)**
iNos	1.43 (0.60)	0.77 (0.11)	1.11 (0.21)	1.53 (0.28)	1.22 (0.34)	1.24 (0.43)
eNos	1.28 (0.36)	0.75 (0.11)	1.06 (0.17)	1.11 (0.22)	1.46 (0.65)	0.66 (0.13)
Hif1	1.04 (0.14)	1.20 (0.13)	1.10 (0.20)	1.05 (0.03)	1.03 (0.11)	0.56 (0.12)*
Fsh_r	1.41 (0.52)	2.66 (0.60)	1.01 (.08)	0.87 (0.80)	1.25 (0.49)	18.22(4.81)*
Lhr	1.38 (0.51)	2.73 (0.87)	1.24 (0.45)	1.88 (0.82)	1.07 (0.18)	1.38 (0.15)
Per1	105 (0.17)	1.15 (0.18)	1.10 (0.20)	2.29 (0.48)*	1.18 (0.29)	0.42 (0.07)*
AchE	1.03 (0.14)	1.09 (0.21)	1.06 (0.14)	0.78 (0.11)	1.08 (0.17)	0.70 (0.09)*
Erα	1.08 (0.17)	0.91 (0.05)	1.39 (0.49)	0.59 (0.07)	0.85 (0.06)	0.62 (0.14)
Bach	0.99 (0.04)	1.00 (0.14)	1.13 (0.25)	0.99 (0.17)	1.09 (0.21)	0.72 (0.16)
P_r	1.12 (0.24)	1.10 (0.38)	1.91 (0.83	1.05 (0.41)	1.39 (0.42)	0.51 (0.28)*
Tnfα	1.06 (0.16)	1.27 (0.17)	1.03 (0.12)	1.40 (0.25)	1.13 (0.23)	1.34 (0.27)

Data are presented as the mean (±sem) fold change from controls.

*Significant at $p < 0.05$ from control.

Table 3.

Fold changes In transcript expression In the pituitary, testes and prostate of males exposed to control or WBV conditions.

Pituitary		Prostate		Testes	
Control	**wbv**	**Control**	**wbv**	**Control**	**wbv**
1.02 (0.11)	0.89 (0.14)	1.36 (0.45)	1.50 (0.39)	1.03 (0.47)	1.25 (0.66)
1.04 (0.13)	1.29 (0.26)	1.15 (0.28)	0.45(0.14) *	1.08 (0.20)	1.02 (0.18)
0.91 (0.21)	1.41 (0.45)			1.45 (0.08)	1.61 (0.31)
1.09 (0.19)	0.34 (0.06) *	1.20 (0.24)	1.23 (0.45)	1.08 (0.18)	0.86 (0.14)
1.38 (0.36)	0.70 (0.15)	0.98(0.18)	0.57 (0.23)	1.22 (0.35)	1.45 (0.27)
0.91 (0.29)	1.89 (.73)	1.03 (0.13)	1.86 (0.54)	1.27 (0.31)	0.99 (0.24)
1.56 (0.80)	1.31 (0.41)	1.37 (0.77)	0.41 (0.27)	1.24 (0.36)	1.15 (0.22)
1.07 (0.20)	1.25(0.48)	1.37 (0.29)	0.33 (0.14) *	1.14 (0.26)	1.63 (0.20)
1.03 (0.19)	1.04 (0.15)	1.34 (0.40)	1.23 (0.17)	1.29 (0.32)	1.01 (0.23)
1.21 (0.40)	1.09 (0.20)	1.04 (0.16)	0.69 (0.23)	0.89 (0.14)	1.32 (0.22)
1.03 (0.12)	1.01 (0.10)	1.08 (0.24)	0.81 (0.23)	1.02 (0.09)	3.03 (0.79) *
		1.05 (0.09)	0.78 (0.11)	1.06 (0.16)	1.13 (0.25)

Data are presented as the mean (±sem) fold change from controls.

* Significant from control $p < 0.05$