in C57BL/6 mice (*n* = 8, ~3-wk-old females) to refine the study by determining how many days post-application skin tissue should be collected to see peak inflammation. Nair was applied to 1 flank of each animal for 2 min, and skin tissue was collected at 3, 5, 7, and 10 d post-application, with the contralateral flank acting as a control. Photos of the skin were taken daily and blind-scored using a modified Draize skin scoring rubric. There were no discernable differences in skin erythema, ulceration, or edema across groups. Total histopathology scores were generated by a dermatopathologist and were based on dermal inflammation, follicular changes, fibroplasia, acanthosis, and changes to the stratum corneum. Histopathology scores of the treated skin were higher at 3 and 5 d post-application, with histopathology scores in the former group being higher with less variability. Based on these results, 3 d post-application of the product was determined to be the peak inflammatory time point for future studies. Further research into the use of depilatory creams on murine skin will help determine best practices for its use in research to promote better animal welfare and improve research outcomes.

P338 Extended Release Buprenorphine Effectively Attenuates Laser-Induced Thermal Hypersensitivity in an Incisional Model in Neonatal Rats

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Research on the effectiveness of neonatal rat analgesic techniques is limited. In this study, we investigate if a newly released, lipid bound, extended-release buprenorphine effectively attenuates thermal hypersensitivity in a neonatal rat incisional pain model. We hypothesized that lipid bound, extended-release buprenorphine would attenuate laser-induced thermal hypersensitivity during postoperative period for rat pups in this model. Male and female postnatal day 5 Sprague Dawley rat pups (n=38) were randomly assigned to 1 of 4 treatment groups: 1) 0.9%NaCl (Saline), 0.1 mL, once SC; 2) sustained release buprenorphine (Bup-SR), 1mg/kg, SC once; 3) low dose extended-release buprenorphine (XLo), 0.65 mg/kg, SC once; 4) high dose extended-release buprenorphine (XHi), 1.3 mg/kg, SC once. Pups were anesthetized with sevoflurane, a 5 mm long skin incision was made over the left lateral thigh, the underlying muscle was dissected, and the skin was closed with surgical glue. Thermal hypersensitivity and clinical observations were evaluated at 1 h prior to surgery/analgesic administration and then subsequently at 1, 4, 8, 24, 48, 72 h. Plasma buprenorphine concentration levels were evaluated at 1, 4, 8, 24, 48, 72 h. Thermal hypersensitivity in the Saline group was significantly enhanced at 1, 4, and 8h. The enhanced thermal hypersensitivity was effectively attenuated in the Bup-SR and XLo groups at all 3 time points. The XHi group showed thermal hypersensitivity attenuation at 1 and 4 h. Plasma buprenorphine concentration for all treatment groups remained near the clinically effective concentration of 1 ng/mL at least 8 h and no abnormal clinical observations were evident. Results indicate that extended-release buprenorphine attenuates laser-induced thermal hypersensitivity during postoperative period for rat pups in this model. Specifically, XLo attenuated postoperative laser-induced thermal hypersensitivity for at least 8h while XHi attenuated post-operative hypersensitivity up to 4 h.

P339 Comparison of Microenvironmental Parameters in Single- and Pair-housed Static Rat Cages

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Social housing and microenvironmental parameters are important considerations when housing rats for research purposes. The objective of this study was to determine if microenvironmental parameters (cage temperature, humidity, NH_3, CO_2, O_2) vary significantly between single- and pair-housed static rat cages housing large (>500g) adult (>6 mo) Crl:CD®(SD)IGS rats. Microenvironmental parameters were measured at 3-timepoints (days 0, 2, 4) over the first week following cage change and were measured within 4 cages each of single- (n=4) and pair-housed (n=8) rats. We hypothesized that pair-housed cages would have higher temperatures, humidity, NH_3, CO_2, and lower O_2 when compared to single-housed cages. There were no significant differences when comparing 1-wk mean cage temperatures (means±1 SD, singles = 76.2±1.6°F, pairs = 76.8°F±1.4°F), humidity (singles = 59.2±15.5%, pairs = 54.7±6.4%), NH_3 (singles = 82.5±110.7 ppm, pairs = 16.8±20.7 ppm), CO_2 (singles = 0.24±0.17%, pairs = 0.17±0.05%), and O_2 (singles = 20.7±0.2%, pairs = 20.7±0.1%) (*P* values > 0.05). Although 1-wk means were not significantly different, at the final timepoint a paradoxical effect was noted as single-housed cages had significantly higher NH_3 (208.3±39.2ppm) and CO_2 (0.4±0.2%) and lower O_2 (20.5±0%) when compared to pair-housed cages (NH_3 = 40.0±10.5ppm, CO_2 = 0.2±0.2%, O_2 = 20.6±0.1%) (*P* values < 0.05). These data reject our hypothesis that pair-housed cages would have higher temperatures, humidity, NH_3, CO_2, and lower O_2 compared to single-housed cages. This study further supports the recommendation for social housing of rats whenever possible unless contraindicated for research or medical purposes.

P340 Refinement of a Traditional Hormone Treatment to Improve Ovulation Rates in Wild Type C57BL/6 Donors for Pronuclear Injection (PNI)

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Pronuclear injection (PNI) is the most widely used procedure to transfer genetic material in the mouse. PNI requires large numbers of high-quality zygotes routinely collected from juvenile 3-4-wk-old donors superovulated with 5IU pregnant mare serum gonadotropin (PMSG) + 5IU human chorionic gonadotropin (hCG). Building on the concept of the 3Rs to reduce, refine, and replace, we hypothesized that using larger amounts of hormones in older pubertal females may improve zygote number and quality while using fewer embryo donors. Two hormone treatments were evaluated: 7.5IU-PMSG/5IU-hCG (7.5-P/5-CG) and 7.5IU-PMSG/7.5IU-hCG (7.5-P/7.5-CG) in 4-, 5-, and 6-wk-old C57BL/6NTac females (*n* = 5/group). The standard 5IU-PMSG + 5IU-hCG (5-P/5-CG) dose was used as control treatment. We first examined number of oocytes and found that 7.5-P/5-CG improved the number of oocytes/female in 4-, 5-, and 6-wk-olds (43.2±14.6, 46.2±14.9, and 27.0±3.7) versus the control (36.8±9.5, 27±5.2, and 21±7), respectively. The 7.5-P/7.5-CG treatment increased the number of oocytes produced in 4-wk-old females (51.6±10.5), but not in the other 2 age groups. Additionally, the number of injectable zygotes produced in each treatment group was evaluated. Females treated with 7.5-P/5-CG produced more injectable zygotes/mouse (42±14.3, 35±15.2, and 17.2±5.7) than the control (34.8±8.6, 19.8±6.3, and 14.4±5) in each age group, respectively. The 7.5-P/7.5-CG treatment did not improve the number of injectable zygotes compared to the 7.5P/5CG group, suggesting that increasing hCG does not provide any benefit to the superovulation protocol. Yield of live offspring/female improved significantly in 5- and 6-wk-olds treated with the 7.5-P/5-CG versus the control (5.5 and 4 vs 0 and 0), respectively. Four-wk-olds treated with 7.5-P/5-CG, or the control, yielded 8.5 pups per female. This study suggests that increasing the amount of PMSG to 7.5IU in older pubertal 5-wk-old donors improves reproductive parameters of total oocytes, injectable zygotes, and live births. According to these results, and in support of the 3Rs principles, refinement of the hormone priming protocol for superovulation helped to reduce the number of donors required for PNI and optimize scientific productivity in our transgenic facility.