P340 Fecal Microbiome Monitoring in Colony and Sentinel Mice

PC Smith[1,2], J Gu[3], C Zhou[3], CJ Zeiss[1], SR Compton[1], PA Cirillo[2], H Wallace[2], J Macy[*1,2]

[1]Comparative Medicine , Yale University, New Haven, CT; [2]Yale Animal Resources Center, Yale University, New Haven, CT; [3]Biostatistics, Yale University, New Haven, CT

To determine if monitoring the fecal microbiome (FM) of soiled bedding sentinels informs changes of FM of colony mice, a study using 6 breeding pairs of in-house reared mice B6.129P2(C)-Tg(Nr5a1-cre)7Lowl/J (SF1) and 6 breeding pairs B6.129P2(C)-Cx3cr1$^{tm2.1(cre/ERT2)Jung}$/J (CXCR), 8 cages of 16 female C57BL/6NCrL (B6) mice, and 8 cages of 16 female Crl:CD1 (CD) commercially acquired mice was performed. Shallow shotgun metagenomic sequencing analysis of individual feces from all mice (56) was performed at 0, 2, 4, 14 and 28 d. At days 49, 63, 77, 91, 105, 119, and 133 soiled bedding combined from 6 cages of SF1 or CXCR were transferred into 2 sentinel cages of B6 or CD and soiled bedding combined from 12 cages of SF1 and CXCR were transferred into 2 B6 or CD sentinel cages. Two control cages of B6 and CD sentinels received no soiled bedding. On days 49, 77, 105 and 133 feces from all mice (56) and pools of feces from SF1 (12 feces) and CXCR (12 feces) mice were collected. Sequence analysis from all samples revealed that sentinels (B6, CD) had higher species richness and diversity than colony mice (SF1, CXCR) and there was no change in alpha diversity over time. For each genotype, beta diversity (inter-individual variation) of FM changed during the first 4 d, stabilized after 14 d, and increased in CXCR from days 28-91. Exposure to soiled bedding from colony mice did not impact alpha diversity but did increase beta diversity of the sentinel mice FM. Genotype had the largest variability with differences between groups segregating by mouse source (in-house vs. vendor), followed by the specific housing cage.

P341 Optimization of Superovulation Hormone Injection Intervals

JA Cayton[*1], C Bethune[1], C Piotter[1], J Walls[1], D Davis[2], H Men[1], CL Franklin[1]

[1]Veterinary Pathobiology, University of Missouri, Columbia, MO; [2]RSCH Core Facilities, University of Missouri, Columbia, MO

Superovulation is routinely used for many murine assisted reproduction procedures. It involves the use of exogenous gonadotropins to induce increased production of mature oocytes. Pregnant mare's serum gonadotropin (PMSG) mimics the oocyte maturation effect produced by endogenous follicle-stimulating hormone. Human chorionic gonadotropin (hCG) mimics the ovulation inducing luteinizing hormone. Current literature suggests use of a 46-48 h interval between PMSG and hCG injections to induce superovulation in mice. In this study, C57BL/6 female mice (n=214) were utilized. We evaluated the number of zygotes and unfertilized oocytes produced using 46- and 48-h time intervals between PMSG (5 IU) and hCG (5 IU) intraperitoneal injections. During this study, our vivarium also removed dark cycle red lighting, which allowed for the evaluation of the impact of this variable on superovulation. There were no significant differences observed in superovulation outcomes between the 46- and 48-h intervals. However, a trend ($P = 0.169$) was observed indicating that a 48-h time interval may yield more zygotes and unfertilized oocytes. Mice in a 12-12 hour light cycle with no dark cycle red light exposure produced significantly ($P = 0.031$) greater zygote and unfertilized oocyte counts at collection. These findings highlight the need for further studies on the many factors that can be exploited to optimize superovulation protocols in rodent research models.

P342 Postsurgical Recovery Assessment of Mice Treated with Extended- or Sustained-Release Buprenorphine

M Saenz[*1,2], EA Bloom-Saldana[2], PT Fueger[2], R Ermel[1], J Finlay[1]

[1]Center for Comparative Medicine, City of Hope, Duarte, CA; [2]Department of Molecular and Cellular Endocrinology, Beckman Research Institute at the City of Hope, Duarte, CA

The principles of *The Guide for the Care and Use of Laboratory Animals* emphasize that pain should be minimized using appropriate analgesia. The extra-label use of sustained-release buprenorphine (SRB) is commonly used in rodents to mitigate mild-to-moderate pain. A new FDA-indexed formulation of a sustained-released buprenorphine, known as extended-release buprenorphine (XRB), has recently become available. Being FDA-indexed, XRB is approved for use in minor animal species because its safety and efficacy has been affirmed by the FDA. However, no studies have directly assessed differences in the recovery of XRB- and SRB-treated mice after a surgical procedure. Thus, the purpose of this study was to quantify post-surgical recovery indices in mice treated with XRB compared to SRB. We hypothesized that post-surgical recovery of mice treated with XRB would be similar to mice treated with SRB. Male and female 13-to-15-wk-old C57Bl/6J mice were anesthetized, treated with either SRB (1 mg/kg, SC, once) or XRB (3.25 mg/kg, SC, once), and underwent a surgical carotid artery catheter implantation (6-8 animals per group). Body weight, body condition, facial pain recognition scale of eye closure, and visual pain-index assessment scores of coat condition, coordination/ posture, and overall condition were recorded daily for 3 d after surgery. As expected, all animals lost weight post-operatively. Post-operative weight loss was similar between groups; body weight declined by 11.7 ± 1.6 and 12.3 ± 0.7% in males and by 7.6 ± 2.2 and 8.1 ± 1.1% in females treated with SRB or XRB, respectively. Total pain-index visual assessment scores indicated both SRB- and XRB-treated animals had mild pain, appearing slightly hunched but able to move about, up to 3 d postoperatively, suggesting these analgesics are effective. There were no significant differences in body condition or individual pain-index visual assessment scores between SRB- or XRB-treated animals. Thus, in the post-surgical recovery assessment of mice, the newly available FDA-indexed XRB is comparable to SRB. Therefore, the availability of XRB increases the options for safe and effective analgesia in mice.

P343 Reducing Inter-operator Variability Using a Novel 3D and Thermal Measurement System when Measuring Subcutaneous Tumors in Mice

A Smith, K Turley, J Steed[*]

Marketing , Fuel3D, Oxford, , United Kingdom

Measurement (length and width) variability between technicians and scientists using calipers to measure subcutaneous tumors implanted in mice is a common issue that can limit the ability to conduct studies. The lack of measurement consistency between operators can cause studies to be delayed or reduce facility capacity. When a single operator must take all measurements during a study to prevent data errors, this may ultimately impact scientific reproducibility. We explore the use of a new tool that uses thermal, 3D, and RGB photographic images to take automated measurements of the tumor length, width, and height to determine a tumor volume and its impact of inter-operator variance. In 3 separate preclinical studies (2 using Balb/C mice, the first with 64 animals in 8 groups and the second with 32 animals in 4 groups and 1 study used C57BL/6 mice with 40 animals across 8 groups), 3 technicians were tasked with capturing measurements of subcutaneous tumors on the flanks of female mice with both callipers and a thermal/3D measurement system on the same day, for the duration of each study protocol. The primary goal was to compare the inter-operator variability of the 2 techniques to see if the thermal/3D system outperformed callipers in the repeatability of measurements between operators. The results were analyised using an interclass correlation (ICC) statistical model