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**OVERVIEW**

**Mohan et al. Contribution of the Breadth and Depth of IACUC Membership to Experimental Design as a Factor in Research Reproducibility, pp. 104-109**

Domain 5: Regulatory Responsibilities; Task 5 - Serve as a member of an IACUC

SUMMARY: The IACUC is a key component at any institution or facility that participates in animal research.  According to the PHS policy, the IACUC should consists of no less than 5 members, and shall include: a DVM, one practicing scientist, a non-scientists, and a non-affiliated member.  A person that meets that requirements of more than one category, may fulfill more than one requirement, however the committee must have at least five members, regardless.

The IACUC has the responsibility to ensure humane treatment of animals, compliance with federal standards, and accountability regarding the use of animal in research.  Additionally, the IACUC has the responsibility to promote high quality scientific research, and thus must ensure rigor in experimental design and reproducibility by addressing factors such as optimization of animal numbers, harm-benefit analysis, and ethical considerations.  As the major funding agency, the NIH has put forth the Initiative to Enhance Research Rigor and Reproducibility, with special emphasis on research design and planning,

There are several limitations with animal research that can lead to issues with reproducibility: study design, standardization (animal care, chemicals, biologicals), environmental variability, reporting discrepancies, and animal physiology. These limitations and variability within with the limitations can degrade the standard and quality of animal research. The IACUC can provide appropriate guidance for experimental design, documentation, and consistency within animal research.

In 2015, the NIH issued a Guide Notice addressing the use of rigor and transparency to increase reproducibility in research. To enhance reproducibility, 4 major areas were addressed:  1) the scientific premise of the proposed research 2) rigorous experimental design for robust and unbiased results 3) considerations or relevant biologic variables 4) authentication of key biologic and chemical resources.  Although the IACUC is primarily focused on the humane use of animals, applications of the 3Rs, and minimization of pain and distress, the IACUC also has the authority to evaluate scientific elements of the protocol.  The IACUC can support reproducibility by educating researchers regarding experimental design, standardization of resources, and assessment of the validity of data.

QUESTIONS

1. What are some standardization issues that may not be considered in study design?

2. What are some reporting discrepancies that many decrease rigor and reproducibility?

3. How does the non-scientific member contribute to rigor and reproducibility?

ANSWERS

1. Administration of biological agents (vectors, antibiotics), chemical reagents, animal care ( analgesics, antibiotics)

2. Under reporting of negative results and un-reported confounding variables and unexpected outcomes.

3. Ensures transparency, adequate consideration of animal alternatives, and complete and understandable description of animal activities.

**ORIGINAL RESEARCH**

***Reproduction***

**Mahabir et al. Reproductive Performance after Unilateral or Bilateral Oviduct Transfer of 2-Cell Embryos in Mice, pp. 110-114**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Embryo transfer is a common procedure in mouse facilities. This can be performed unilaterally or bilaterally though differences in reproductive performance between these methods is unknown. This study retrospectively evaluated pregnancy rates, litter size, birth rates, and the influence of the number of 2-cell embryos transferred from donors on C57BL/6J, C57BL/6N, or an unknown B6 substrain backgrounds to CD1 recipients. The unknown B6 substrains were derived with fewer than 5 generations of backcrossing to a B6 and represented 'unknown' lines routinely submitted for rederivation. A total of 1619 embryo transferred were evaluated with 939 on a B6J background, 421 on a B6N background, and 259 on the representative B6 substrain. All animals received 15-17 embryos either placed into a single uterine horn or evenly divided over both. Pregnancy rate was significantly higher for B6J lines (85% vs 79%) but did not differ for the B6N or B6 substrain lines. The mean number of pups per litter was significantly higher for bilateral transfer in the B6J (6.9 vs 5.9 pups) and the B6N (6.2 vs 5.0 pups) but not the B6 substrains. Additionally, the B6J lines had higher numbers of pups per litter when compared to the B6N lines for both transfer methods. No significant difference was noted in the number of embryos that developed into live pups between the methods for any of the strains evaluated. The authors did note that despite the higher pregnancy rate and larger litter size, bilateral embryo transfer requires an additional incision and tissue manipulation. This could result in more pain and discomfort and should be taken into consideration when deciding on which technique to use.

QUESTIONS

1. True or False. Transuterine migration between uterine horns does not occur in mice following unilateral embryo transfer.

2. Why are outbred stock such as Swiss or CD1 commonly used as recipients for embryo transfer?

ANSWERS

1. True

2. Large litter size and good mothering ability

**Luo et al. Effect of Prepregnancy Obesity on Litter Size in Primiparous Minipigs, pp. 115-123**

Primary Species: Pig (*Sus scrofa*)

Domain 1; K2

SUMMARY: Minipigs have become widely accepted as alternative to mice for reproductive, metabolic, pharmacologic, and toxicologic studies. Minipigs come into sexual maturity at 4 to 5 months versus 3-4 years in NHP. Primiparous minipigs typically have small litters and few weaned piglets. Obesity is associated with increased risk of pregnancy loss. Therefore, this study aimed to evaluate the effects of prepregnancy obesity, using porcine obesity index (POI) and obesity-related hormones (sex hormones and peptide hormones) before ovulation on litter size in 3 breeds of primiparous minipigs (Bama, Tibet, and Wuzhisan).

Results:Prepregnancy body weight and POI increased with age in all 3 breeds. Bama nulliparous minipigs had the highest prepregnancy body weight and POI, while Tibet minipigs had the lowest. There was a significant negative correlation between prepregnancy POI and litter size. Positive correlations emerged between litter size and prepregnancy estradiol, FSH, LH or progesterone in all 3 breeds, except for estradiol in Bama minipigs and FSH and progesterone in Tibet minipigs. Significant negative correlation occurred between prepregnancy prolactin and testosterone and litter size in Tibet and Wuzhishan minipigs. All 3 breeds of minipigs showed significant negative correlation between prepregnancy cortisol and litter size, whereas prepregnancy IGF1, GH, insulin, and leptin were associated with significant positive effects. Litter size was not significantly correlated with prepregnancy levels of glucagon, FT4, or FT3.

QUESTIONS

1. At what age are minipigs sexually mature?

1. 3-4 years
2. 4-5 months
3. 10-12 months
4. 4-5 weeks
5. 2 months

2. What is the effect of prepregnancy obesity on litter size in primiparous minipigs?

1. Reduces litter size
2. Increases litter size
3. Has no effect on litter size

3. What peptide hormone shows negative correlation with litter size?

1. Leptin
2. Insulin
3. IGF-1
4. Growth hormone
5. Cortisol

ANSWERS

1. b

2. a

3. e

***Husbandry***

**Robinson-Junker et al. Out Like a Light? The Effects of a Diurnal Husbandry Schedule on Mouse Sleep and Behavior, pp. 124-133**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: This study examined if the timing of husbandry activities altered sleep patterns of mice and if the presence of more nesting material buffered those alteration. The study used 48 6 week old mice, males and females of C57BL/6, Crl:CDI, and BALB/c backgrounds. Mice were give 1 of 4 different amounts of nesting material (3-12 g), maintained on a 12:12 light cycle, with a red LED light on from 2200-2300 for observation, and individually housed in sleep monitoring apparatuses. The sleep monitoring was done through monitoring of respiration patterns. Mice were also video recorded. Mice were acclimated first, on the opposite schedule of disturbance than first tested on. After 7 days testing mice were handled briefly and switched to the second schedule of disturbance. Daily disturbance included monitoring of food, water, and health, as well as daily room maintenance. In addition to sleep monitoring, ethograms and nesting scores were also recorded.

The only significant effect on sleep time was strain specific, with C57BL/6 spending less time sleeping. Sex, strain, and night vs day disturbance showed statically significant interactions regarding differences in day and night sleeping. Strain and sex of mice were shown to affect behavior. All mice were less active during lights on times and mice with the most nesting material were less active than mice will less nesting material. Potentially the higher amount of nesting material buffered the vibrations that detected activity in these mice. Increased nesting material showed differences in sleep immediately before lights came on and immediately after the light time disturbance. Changes in nesting material amounts did not change the time in nests or score of nests over the study, although more time was spent in higher scoring nests. Mice with dark time disturbance exhibited less behavior during light times. Overall mice with light time disturbance didn’t show less sleep than those with dark time disturbances. The more days out mice were from cage changes, the more their sleep progressed later into the dark cycle, but only daytime cage changes affected these sleep patterns.

Overall the study showed that the timing of daily disturbances of the mice did not impact their sleep patterns, but the mice did respond differently to the timing of the disturbances.

QUESTIONS

1. How did the amount of nesting material provided compare to what is individually portioned commercially?

2. How does the individual housing of these mice potentially impact results?

ANSWERS

1. These mice were provided 3, 6, 9, and 12 g. Various commercial nestlets provide 1.4-3 g material.

2. Individually housing mice removes the interaction between mice and mice disturbing their cage mates sleep patterns.

**Lutz and Brown. Porches as Enrichment for Singly Housed Cynomolgus Macaques (*Macaca fascicularis*), pp. 134-137**

Domain 4

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Environmental enrichment can be classified into five different categories: social which includes housing in groups or pairs; sensory such as watching television or listening to the radio; nutritional which includes offering novel or special food items; occupational which includes food puzzles; and physical which includes providing toys or additions to the cage. Porches are a type of physical enrichment that can be attached to the front of a cage and provide the animal with access to additional space as well as providing the animal with an option to better see neighbors and allow for additional social interaction. Since not all enrichment has the same benefits for each animal, the effectiveness of the enrichment item must be assessed by determining if it decreases levels of abnormal behavior, increases species-typical behavior, and determining how much animals use it. In addition, identifying which animals (i.e. temperament, age, sex, etc) would benefit the most from particular enrichment items is important. This study was to assess the effectiveness of porches as an enrichment device for singly housed Cynomolgus macaques and identify which animals would benefit most from porches.

Materials: 18 Cynomolgus macaques, 9 male and 9 female aged 3.5-6.5 years old were used in this study. All animals were singly housed. The porches were made of stainless steel mesh and were attached to the front of the cage. Animals were videotaped for 15 minutes daily for 3 days per week for 3 weeks. Week 1: no porches were provided. Week 2: porch was provided the entire time. Week 3: removal of porch. Prior to starting this study animals were classified by temperament (bold, intermediate or inhibited) based on the average time it took the animal to touch a novel object (novel object temperament testing). 8 animals were categorized as bold and 10 were intermediate.

Results: Animals showed more active behaviors (standing up, sitting down or moving around cage) before exposure to the porches than during or after. They rested (sitting or standing motionless for at least 2 sec) more during porch exposure. They consumed more food and water before than after porch exposure. Other behaviors (pacing, scratching, yawning and self-directed manipulation) occurred at the same frequency across test conditions. The porches were used for approximately 75% of the observation time. Males used porches more than females. Animals with intermediate temperaments were less likely to use the porches when they were located in lower cages.

Discussion: Porches were effective environmental enrichment tools for cynomolgus macaques. They were used more frequently than other physical enrichment items such as toys.

QUESTIONS

1.   What are the 5 different categories of environmental enrichment according to this article?

2.   (T/F) The aim of this study was to determine if singly housed cynomolgus macaques benefited from the addition of porches to their cages.

3.   (T/F) Female cynomolgus macaques used the porches more than males in this study.

ANSWERS

1.   Social, nutritional, sensory,  occupational and physical

2. True

3.  False

***Management***

**Ball et al. Evaluation of Extended Sanitation Interval for Cage Top Components in Individually Ventilated Mouse Cages, pp. 138-142**

Domain 4: Animal care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The frequency of changing and sanitation of rodent cage components to produce an appropriate microenvironment for the housed animals is a subject of debate. Bedding and cage bottoms can become soiled rapidly and require changing on 1- or 2-week intervals, whereas feeders and lids must be changed frequently enough to ensure animal welfare.

Aim: The microbial load on cage top surfaces and air quality within cages was assessed to determine whether the continual use of the wire lid and filter top would over time result in an increase in bacterial contamination of the cage tops or clogging of the filters resulting in an increase in ammonia.  
  
Method: Mice were housed in groups of 4 or 5 in IVCs (size 9 Thoren) on paper bedding with a cage ventilation rate of 30 to 60 cage volume air changes per hour. Daily welfare checks were carried out without removing the cages from the rack. After 2 weeks, cages were removed from the racks for changing. This was continued for an 8-week period.

NIH Swiss mice were used in the microbiology and welfare assessment. Unoccupied control cages containing bedding and feed were set up at different locations on the rack. Every two weeks the cage bottoms and bedding were changed and 2 sites on the filter tops and 2 sites on the wire lid were swabbed for culture. Aerobic bacterial colonies were counted after 24h of incubation. A clinical veterinarian scored the animals’ appearance and behavior as normal or abnormal.

Retired mice on B6 and C backgrounds were used in the ammonia monitoring study. Mouse cages were fitted with either new filters or filters in active use in the facility. The ammonia level was tested after removal of the cage from the rack. Test strips in which the color changed at 5, 10, 20, 50 and 100 ppm were introduced through a grommet at the back of the cage before the cage was opened. Positive control tests were done at each assay point using household ammonia applied to paper bedding in an empty cage.  
  
The Friedman test was used to compare microbial load at 4 time-points, applying a P value of 0.05.

Results: The microbial load ranged from 0 – 1 CFUs in control and 0 – 140 CFUs in unoccupied cages. There was no significant difference between the microbial loads at either location on the wire lid or food hopper and a significant decrease in microbial load between the 2-week time-point and later time-points. No welfare concerns were reported.

After 2, 4, 6 and 8 weeks of housing, cages resulted in either no or very slight color change (less than 5ppm). There were no differences in intracage ammonia levels according to sex or filter status.

Conclusion: A change interval of 8 weeks for wire lids is justified in light of the lack of significant increase in microbial load and low microbial load on filters.

QUESTIONS

1. What is the maximum amount of ammonia exposure permitted in people working with rodents?

a. 10 ppm

b. 25 ppm

c. 45 ppm

d. 85 ppm

2. T/F. Increased microenvironmental relative humidity in rodent cages may lower intracage ammonia concentrations.

ANSWERS

1. b. 25 ppm

2. F

**Baker and Hickman. Bias in Rating of Rodent Distress during Anesthesia Induction for Anesthesia Compared with Euthanasia, pp. 143-156**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The authors think that personal expectation and a person’s capacity for empathy can modify the evaluation of animal welfare in mice and rats. To prove that, they designed a study to explore how the perception bias of laboratory animal professionals affects their evaluation of the wellbeing of animals that are used in research.

They showed recordings of the induction phase of the anesthesia to multiple volunteers. Two groups of participants were  told that they would be observing euthanasia of rats or mice. The second 2 groups of participants were told that they would be observing the euthanasia of rats or mice. However, all groups watched the same 4 rats videos or the same 4 mouse videos, regardless of their assignment to treatment groups.

For the study, 4 rats and 4mice were individually anesthetized or euthanized using 1 of 4 methods of anesthesia or euthanasia. The participants were told that they will be asked to rate the attempts by the animal to escape, fearfulness, respiratory distress, other distress, and the appropriateness of the method of anesthesia or euthanasia.

The results of the study suggest that a perception bias was present in the persons sampled. It is noteworthy that, overall, participants provided the 4 mouse videos rated the mouse’s experience more negatively when they were told that the mouse was being euthanized as compared with being anesthetized. A similar trend was present in the responses of the participants provided the 4 rat videos, although the overall mean scores did not differ significantly. Observers reported less potential distress for rats and this might be due to a variety of reasons, including their large size, which made it easier to evaluate the animal on the recording. In addition, species bias might be present, if the participants were more comfortable with the behaviors of rats.

In summary, the findings support why controlled studies that are objectively focused on the animal and a combination of its behavior, physiology, and affective state provide stronger evidence for evaluating potential pain and distress than studies that rely heavily on subjective-and potentially biased- interpretation of behaviors alone.

QUESTIONS

1. Give a definition to the term euthanasia

2. Selection an appropriate method of euthanasia involves evaluating multiple criteria such as:

a. Wellbeing of the animal

b. Intended use of the animal after death

c. Physical and psychologic safety of the observer or operator

d. All

3. Perception bias is well recognized as a phenomenon in the human medical profession, especially with regards to traits such as:

a. Race

b. Socioeconomic status

c. Age

d. All

ANSWERS

1. Euthanasia is defined as “ending of the life of an individual animal in a way that minimizes or eliminates pain or distress”.

2. d is true

3. d is true

**Gerwin et al. Using a Time-Driven Activity-Based Costing Model to Determine the Actual Cost of Services Provided by a Transgenic Core, pp. 157-160**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Laboratory animal programs and core facilities usually base rates of services on cost estimates that often divide indirect costs over number of labor hours. This generalized model may lead to inaccurate fees for different activities. There has been a shift towards using activity-based costing (ABC), which more specifically calculates the cost of a given task. A time-driven ABC model utilizes the time required to perform an activity (=’unit time’) and employee cost per minute (‘=’unit cost of supplying capacity’). This model was used to determine the labor and cost of a Transgenic Core Resource Laboratory at the authors’ institution. For 1 week, employees recorded the time spent on *in vitro* fertilization (IVF), embryo transfer (ET), microinjection of DNA or embryonic stem cells, and rederivation. Supplying capacity was determined by dividing salary of employees by ‘practical capacity,’ defined as 80% of total work time. ‘Unit cost,’ defined as the amount of money each employee costs the institution per minute, was also evaluated. Actual cost of the Core services was calculated based on operational costs of the Core, including *per dime* rates, cost of animal purchase, and colony management costs. This was compared to the service fees charged to the users, which is lower due to subsidies from the institution.

Results showed that IVF accounted for ~50% of total capacity in both time and cost. Actual work capacity was higher than practical capacity, indicating that the Core employees were operating above capacity and would benefit from an additional employee to meet growing demands. Results showed that the institutional subsidies covered 49% of Transgenic Core services, which was close to the institutional target of 53%. The time-driven ABC model is flexible and can be adjusted to reflect changes in salaries and time for new services and employees. It can be used to better schedule employee activities, evaluate program efficiency, and more accurately price new services, such as gene editing through CRISPR.

QUESTIONS

1. Which of the following is not an indirect cost?
   1. Energy bills
   2. Taxes
   3. Membership fees for professional societies
   4. Director’s salary
   5. Water bills
2. Which is not a basic principle of animal research facilities finances, according to the NIH *Cost Analysis and Rate Setting Manual for Animal Resource Facilities*?

a.   Billing rates should be based on costs

b.   The objective is to generate a surplus to cover any emergency costs that may arise

c.  All costs associated with providing an animal service should be included in the total cost of each service

d.  The costs must be treated consistently as indirect or direct costs

e.  Billing units should logically represent the service provided

ANSWERS

1. c
2. b

**Devan et al. Improvement of Vivarium Biodecontamination through Data-acquisition Systems and Automation, pp. 161-172**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Domain 4: Animal Care

K3 - Methods of sterilization, sanitation, and decontamination

K4 - Quality assurance techniques for animal care-related equipment (e.g., verification of effective cage sanitation) and supplies (e.g., water, food, bedding)

SUMMARY: Eliminating pathogens from research facilities can be challenging. Traditionally, formalin fumigation was commonly used to decontaminate research rooms after outbreaks and while it was effective, it had safety concerns. These authors studied the combination of vaporized hydrogen peroxide (VPH) and chlorine dioxide gas. The process was able to be applied to specific rooms and was validated.

QUESTIONS

1.  What is the name of the biologic agent used to validate decontamination?

2.   In addition to safety, what is another appealing reason to decontaminate with VPH?

ANSWERS

1.  *Geobacillus stearothermophilus*

2.  Non-corrosive

***Animal Health Surveillance***

**Nashat et al. Comparison of Diagnostic Methods and Sampling Sites for the Detection of *Demodex musculi*, pp. 173-186**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Mouse (*Mus musculus*)

SUMMARY

Goal:Identify antemortem diagnostics that are sensitive, minimally invasive, easy to perform, and economical for *Demodex*mites in mice, specifically *D. musculi*. Focused on traditional microscopic methods of parasite detection and PCR.

Background: *Demodex*spp. mites are mammalian microfauna that typically inhabit the pilosebaceous unit of healthy animals. They are found in low numbers in normal skin and adnexa and usually do not cause clinical manifestations; in immunocompromised hosts, they can initiate dermatologic disease. Demodicid mites tend to be host-specific. Relatively few instances of *Demodex*mite infestation have been reported in laboratory mice, but all occurred in immunocompromised strains and, only *Demodex musculi*was identified. Diagnosis of *D. musculi*is challenging because the mites are microscopic (200μm), transparent, and live within hair follicles. These mites can infest most areas of densely haired skin. In dogs, deep skin scrape (DSS) is the ‘gold standard’ for the detection of *D. canis*, but fur pluck (FP) with trichoscopy (microscopic evaluation of hairs placed in mineral oil on a microscope slide), can be used in heavy infestations. FP is useful for anatomic sites such as the face and interdigital.

Methods: Mouse colony: A colony of immunocompromised transgenic mice (TRP1/TCR mice) with a recombination activation gene 1 (RAG1) deficiency presented with exophthalmia and pruritus - B6.Cg-Rag1tm1Mom Tyrp1B-W Tg(Tcra,Tcrb)9Rest. Contain 3transgenes: the tyrosinase-related protein 1 transgene (Tcra,Tcrb) 9Rest, the white-based brown radiation-induced mutation of tyrosinase-related protein (Tyrp1B-W), and a *Rag1*mutation (Rag1tm1Mom). The *Rag1*-null mutation inactivates an enzyme critical to normal lymphocyte development, which results in defective adaptive immunity due to the absence of functional mature T and B lymphocytes. Opportunistic bacterial infections and an infestation with *D. musculi* were identified on histopathology.

Diagnostics Assessed: Superficial skin scrape (SSS), tape impression (TI), fur pluck (FP), and deep skin scrape (DSS).

Sites: Face, interscapular region (IS), caudal dorsum (CD), and caudal ventrum (CV).

PCR: Fresh–frozen and formalin-fixed skin and samples from TI tests.

Results:

* + Highest to lowest overall detection rate by test type:  DSS > FP > SSS > TI
  + DSS detected significantly more mites per sample compared with all other methods
  + IS > CV and CD had similar detection rates; lowest detection rates were for TI–face and FP–face
  + PCR detected *Demodex*mites in frozen skin, formalin-fixed skin, and as individual *D. musculi*mites preserved on tape
  + DSS from either site was equivalent to PCR analysis in detecting infested mice (100%)
  + In FP samples, the opisthosoma (caudal region) of the mite is approximately the same width as a mouse hair bulb, making it challenging to detect mites present in hair clusters.
  + DSS was the easiest method to evaluate due to decreased debris and lack of hair bulbs
  + Mites were easiest to identify in fresh, oil-based samples because they were often mobile, and their internal organs were visible
  + Adult female mites were longer than males; More female than males (likely related to their tendency for arrhenotoky, a form of parthenogenesis)
  + Eggs and larvae were visible on oil-based tests (DSS and FP); FP > DSS for detecting eggs or larvae.

Discussion/Summary: Results indicate that DSS, when combined with FP, at 2 different sites (IS and CV) yields 100% detection for *D. musculi*in a moderately infested mouse strain. When DSS are performed correctly in most species, the epidermis is scraped down to the dermis, and bleeding occurs with sufficient sampling depth. In contrast, when DSS samples are collected in mice, the skin does not bleed, likely because the murine dermis is poorly vascularized. In domestic cats, *Demodex*mites have been identified through coproscopy (fecal flotation), presumably because cats are avid groomers. SSS and TI are often used to detect other parasites in mice, such as fur mites and select pinworm species (e.g. *Syphacia obvelata*). A structure that was helpful in identification of mites in oil-based tests was a small, opaque structure called the guanine concretion, which is the deposition of a pigmented nitrogenous waste product in the opisthosoma. The guanine concretion was refractive, making it identifiable with subtle changes in light or depth of focus and was observed in most live or recently dead mites.

QUESTIONS

1. What is the “gold standard” of *Demodex* detection in dogs?
2. In what animal, can *Demodex* be detected via fecal float?
3. In terms of *Demodex*, what is the guanine concretion?

ANSWERS

1. Deep skin scrape
2. Domestic cats
3. Deposition of a pigmented nitrogenous waste product in the opisthosoma, making microscopic identification easier

***Anesthesia***

**Oliver et al. Using Cageside Measures to Evaluate Analgesic Efficacy in Mice (*Mus musculus*) after Surgery, pp. 186-201**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The authors identified a need to further characterize the efficacy of the most relied-on analgesics and ensure that mouse welfare and the scientific validity of the models they provide are not compromised. They sought to compare the efficacy of buprenorphine and carprofen, alone and in combination with postoperative analgesics in mice. They used a combination of assessment measures to ensure that they would capture multiple aspects of pain. The authors included 2 pain scoring systems, nesting consolidation and grooming transfer tests, in the interest of promoting the growth of practical cageside measures that can be used in real time and with minimal resources. Their goals were to determine the best-practice postoperative analgesia protocol for commonly used analgesics, buprenorphine and carprofen, by using multiple postoperative assessments and to establish the validity and reliability of 2 practical, cageside measures for detecting postoperative pain in mice.

Adult male and female C57BL/6 and CD1 mice were used in this study. A fractional factorial design of 4 (control and analgesic treatments) × 2 (sex) × 2 (strain/stock) × 3 (housing schemes) × 2 (replicates), for a total of 96 animals, was used. Both strain/stock and sexes of mice were randomly assigned into one of 4 treatment groups (saline, buprenorphine, carprofen or multimodal) and one of 3 housing schemes (pair, single or single with nest). Each mouse serves as its own control, thus undergoing multiple pain assessment tests at baseline, anesthesia–analgesia, and surgery conditions across multiple time points. At each time point, mice underwent evaluation for nesting consolidation, grooming transfer, mechanical threshold, and locomotor activity. Mice received 1 of 4 analgesic treatments: saline (0.1 mL SC every 12 h for 48 h; 0.9% Sodium Chloride Injection); buprenorphine hydrochloride (0.1 mg/kg SC every 12 h for 48 h); carprofen (30 mg/kg in drinking water provided 24 h prior to anesthesia–analgesia or surgery and 48 h post-anesthesia and surgery); or multimodal treatment consisting of buprenorphine hydrochloride (0.1 mg/kg SC every 12 h for 48 h) and carprofen (30 mg/kg in drinking water provided 24 h prior to anesthesia– analgesia or surgery and 48 h post-anesthesia and surgery).

Mice treated with a multimodal regimen have the most analgesic coverage over the 48 h after surgery, whereas buprenorphine as a single agent showed slightly less coverage, and the carprofen and saline-control groups indicated pain throughout most, if not all, of the postoperative time points. However, multimodally treated animals still showed evidence of pain in the grooming transfer test from 12 through 36 h after surgery. Multimodal analgesia was associated with a significant decrease in body weight (1.5 g) at 24 and 48 h after anesthesia–analgesia, independent of a pain stimulus. Only minor differences were observed between sexes and between C57BL/6 and CD1 mice. The nesting consolidation and grooming transfer tests were found to be valid and practical cageside assessments that provide robust pain measures independent of mouse strain or stock, sex, or housing variables.

QUESTIONS

1.  Which analgesic is known to cause hyperactivity in mice?

a.   Meloxicam

b.   Carprofen

c.   Buprenorphine

d.  Ketoprofen

2.   Which analgesic has been associated to pica in rats?

a.  Meloxicam

b.  Carprofen

c.  Buprenorphine

d.   Ketoprofen

ANSWERS

1.  c

2.   c

**Smith et al. Comparison of Etomidate, Benzocaine, and MS222 Anesthesia with and without Subsequent Flunixin Meglumine Analgesia in African Clawed Frogs (*Xenopus laevis*), pp. 202-209**

Domain 2: Management of Pain and Distress; T1 - Recognize pain and/or distress; T2 - Minimize or eliminate pain and/or distress; T3 - Administration of anesthesia

Secondary Species: African Clawed Frogs (*Xenopus laevis*).

SUMMARY: *Xenopus* frogs have been used for oocyte collection, the vocal pathway, cardiac morphology, cranial osteogenesis, suture morphology, spinal cord regeneration, and androgen regulation. Tricaine (MS222) is the “gold standard” in *Xenopus* immersion anesthesia, but drawbacks include respiratory irritation to personnel, and the need to buffer the solution. The goal of this study was to determine if etomidate or benzocaine produced similar anesthetic effects to MS222 in African clawed frogs (avoiding the occupational health and safety risks of MS222) and characterize the analgesic properties of flunixin in combination with etomidate, benzocaine, or MS222. Frogs were split into 12 groups and exposed to different anesthetic regimens (benzocaine (0.1%, 0.5%, 1%), etomidate (15, 22.5, 30 mg/L), MS222 2.0 g/L) with or without the presence of flunixin (injected into the dorsal lymph sac). A surgical incision was created in the lower abdomen. Pain control was tested by applying a drop of acetic acid in increasing concentrations to the dorsum until the frog exhibited a wiping response to irritation. Etomidate (22.5 mg/L), benzocaine (0.1%), and MS222 (2.0 g/L) were all able to produce a surgical plane of anesthesia. The injection of flunixin provided some anesthetic relief but resulted in mild-moderate myocyte denegation and necrosis, and death of 5/12 frogs at the higher tested dose (50 mg/kg).

QUESTIONS

1.  Euthanasia solutions such as sodium pentobarbital can be injected by what 3 routes in amphibians?

2.  2 other methods of euthanasia acceptable by the AVMA are?

3.   How were *X. laeveis* originally used?

ANSWERS

1.   IV, into the coelom, into the dorsal lymph sacs

2.  Euthanasia methods:

a.  Immersion or injection of buffered MS222

b.  Immersion or topical application of benzocaine

3.  In pregnancy assays. Injection of a pregnant woman’s urine into the dorsal lymph sac of a female *X. laevi*s caused the frog to begin laying eggs.