**Journal of the American Association for Laboratory Animal Science**

Volume 59, Number 6, November 2020

**OVERVIEW**

**Budda and Pritt. Evaluating IACUCs: Previous Research and Future Directions, pp. 656-664**

Domain 5

SUMMARY: Despite the Institutional Animal Care and Use Committee’s (IACUC) essential role in promoting animal welfare standards and ensuring compliance to federal regulations care in institutions, there is extensive leeway given to individual committees when carrying out the functions of IACUC. There is little published data to provide established procedures for administrators to evaluate IACUC performance and prioritize quality improvement. With MEDLINE database, a literature search was performed using a combination of medical subject heading (MeSH) terms, such as animal care committee, institutional biosafety committee (IBC), research, institutional review board (IRB), and IACUC, to identify methods previously used to assess IACUCs. Out of the 105 publications found, only 17 publications pertaining to IACUC were identified. The 105 identified publications in literature included methods of evaluation that may be helpful to IACUC administrators. A majority of these publications noted the use of qualitative methods, such as questionnaires, observations, interviews, self-assessments, and surveys. The benefits of continuous data collection for evaluating IACUC performance include the ability to educate committee members, researchers, institutional leadership and the public about the function and effectiveness of IACUC; could improve efficiency and public perception of IACUC functions; and could reveal areas requiring attention or suggest novel approaches to remediating deficits in IACUC performance. In conclusion, the authors identified a lack of emphasis on evaluating IACUC performance, compared to extensive literature emphasis in IRB performance. This discrepancy could potentially be due to IRB and human research protection programs access to more resources and financial aid compared to IBCs and IACUCs. Based on these findings, there is a need to establish more universal criteria for IACUC performance evaluations and provisions to aid IACUC administrators.

QUESTIONS

1. Which of the following functions is NOT typically assigned under IACUC?
   1. Reviewing all research proposals
   2. Inspecting animal facilities and animal use areas
   3. Reviewing the institution’s program for animal care and use
   4. Investigating concerns involving the care and use of animals
   5. All of the above are assigned functions of IACUC
2. What source does not list the functions for Institutional. Animal Care and Use Committee?
   1. Animal Welfare Act
   2. Public Health Service’s Policy on Humane. Care and Use of Laboratory Animals
   3. The Guide for the Care & Use of Laboratory Animals
   4. All of the above

ANSWERS

1. a, responsible to review protocol involving animal based models
2. d, all discuss the function of IACUC

**Turner. Noise and Vibration in the Vivarium: Recommendations for Developing a Measurement Plan, pp. 665-672**

Domain 2

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Noise, ultrasonic noise, and vibration are confounding variables that impact research and animal welfare, especially if we think in rodents, as they rely heavily on their senses of hearing and touch/vibration. The Design REquirements Manual (DRM) suggests that vivarium environments remain below NC45, however, the NC (noise criterion or noise rating curve) measure of room noise is designed for human hearing only. The DRM Manual lists no specific vibration level to avoid for animals. This lack of information means that researchers have limited guidance on how to deal with noise and vibration concerns.

This paper described commended practices that consist of 4 items that are conservative, and facilities should generally have little problem achieving these standards with minimal resources and planning.

Facilities should have a written noise and vibration plan (NVP) on which methods and frequency for the measurements, and how and when those measurements are communicated to stakeholders should be described. This will help to create a climate of care and attention to these important variables. The NVP should include some annual training on the impacts of noise and vibration on research animals.

Facilities should also conduct an annual noise and vibration assessment in order to periodically review the written NVP and to consider whether noise and vibration concerns have emerged during the last year and what could be done to address them. Annual assessments could include measurements from the macroenvironment and from the cage-level microenvironment to determine which macroenvironmental noises and vibrations are reaching the animal's microenvironment.

Noise levels inside the cage should be maintained below 70 db SPL. Continuous noise levels of 70 db or greater could be expected to affect animal in a range of ways. This level of background noise might mask vocalizations or other communications among animals. Additional evidence has demonstrated that chronic exposure to 70 dB SPL noise can affect auditory structures and functions ranging from the cochlea to the cortex, with changes in molecular and anatomic systems. In the experience of the authors, the presence of food and bedding serve to lower the noise reverberation and the levels of noise in the animal cage.

Vibration levels inside the cage should be maintained below 0.025 g. In addition to the concern that chronic vibration presents a chronic stressor to research animals, several studies have found significant biological and behavioral changes in animals exposed to chronic vibration. As with noise, enrichment items and bedding can also help to limit/absorb some of the vibration.

In conclusion, noise and vibration are ubiquitous and can introduce unrecognized variability to our research models.

QUESTIONS

1. What do you understand by the term "condition monitoring"?

2. What type of changes in the animals can we observed as consequence of high noise and vibration?

3. Which is the most commonly reported finding as a consequence of high vibration levels in the animals?

4. At which frequency can hear rodents?

ANSWERS

1. Is a process by which the mechanical health of an equipment is evaluated using the noise and vibration serving as predictors of mechanical faults.

2. Changes in breeding, behavior suggestive of the presence of a stressor, changes in the general health, or changes reported by the investigators in their study results that might be the product of an environmental stressor.

3. Elevated corticosterone levels.

4. Rodents can hear ultrasonic frequencies above the human upper limit of 20 kHz.

**ORIGINAL RESEARCH**

***Biology***

**Megarani et al. Comparative Morphology and Morphometry of Blood Cells in Zebrafish (*Danio rerio*), Common Carp (*Cyprinus carpio carpio*), and Tilapia (*Oreochromis niloticus*), pp. 673-680**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions; K1 - diagnostic procedures, b. clinical pathology

Primary Species: Zebrafish (Danio rerio)

Tertiary Species: Other Fish

SUMMARY: This journal article compared the morphology and morphometry of blood cells in zebrafish, common carp and tilapia. Zebrafish are one of the more commonly used laboratory animal species and its popularity is growing.  Understanding the differences in cytology may be an invaluable clinical tool.

In summary, the zebrafish blood cells were oval, disk-shaped where the nuclei were more elongated than the carp or tilapia.  All three species stained light blue to lightly basophilic. Interestingly, this article refers to neutrophils as “neutrophils” while other articles have used heterophils. Eosinophils of zebrafish were different than those of carp and tilapia. Basophils were not identified in zebrafish.

QUESTIONS

1.  This article revealed that zebrafish do not have an equivalent

a.  Eosinophil

b.  Basophil

c.   Monocyte

d.  Platelets

2.  According to this article zebrafish belong in the family

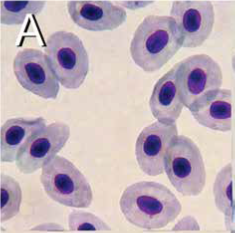
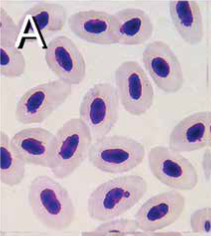
a.  Cyprinidae

b.  Danionidae

c.   Leptobarbidae

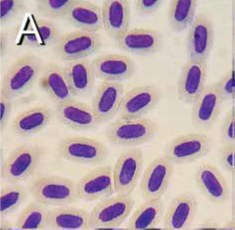
d.  Paedocyprididae

3.  Which image more likely represents zebrafish erythrocytes?

**A**

**B**



**C**

ANSWERS

1.  b

2. a

3.  c

***Reproduction***

**Strelchenko et al. Cryopreservation of Mauritian Cynomolgus Macaque (*Macaca fascicularis*) Sperm in Chemically Defined Medium, pp. 681-686**

Domain 3

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: MCMs (Mauritian Cynomolgus macaques) was used in this study as these are a subspecies of Macaca fascicularis that are descended from a small founder population and have a very limited MHC diversity consisting of only 7 common haplotypes (M1-M7) and this unique features makes them an exceptional model for AIDS pathogenesis, novel vaccine development and stem cell therapies and as such cryobanking MCM sperm to effectively preserve valuable genetic resources, facilitate IVF experiments and application of genome editing strategies.

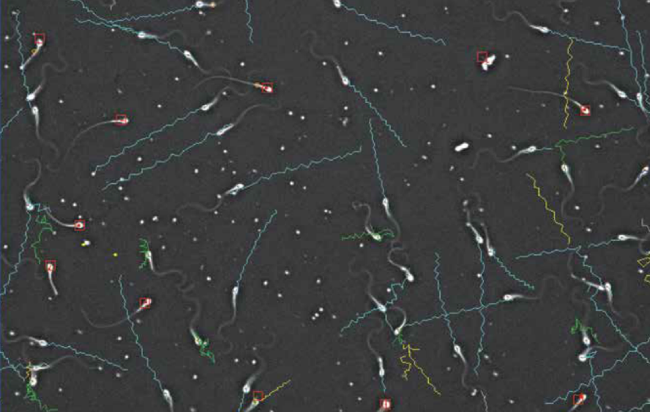
As mammalian sperm cryopreservation is usually accompanied by decrease sperm quality, motility, survival and ability of the sperm to fertilize. This study was conducted to develop a protocol for freezing MCM sperm using a chemically defined extender and checking recovered sperm functionality  via flowcytometry, sperm kinematics and in vitro fertilization potential via ICSI of MCM oocytes.

Results:

1.   Extender composed of PIPES (piperazine-N,N’-bis(2-ethanesulfonic acid) and TES (2-[[1,3-dihydroxy-2- (hydroxymethyl)propan-2-yl]amino] ethanesulfonic acid) buffering agents, trehalose, raffinose, glucose, amino acids and human albumin were used instead of the common egg yolk and tris buffers which changes pH and are thought to contribute to reduced sperm survival rates as the major purpose of extenders is to protect sperm from their own toxic byproducts and to remove inorganic ingredients that negatively affect cryopreservation. In addition, given that osmolarity is also a major factor, extender was modulated by 5% glycerol and sperm viability was measured by flow cytometric analysis. Based on this, an osmolarity of 310 mOsm provided the best sperm viability after thawing, while a higher osmolarity markedly decreased sperm viability.

2.   Effect of Cryoprotectants was also evaluated on sperm viability using either ethylene glycol (1.5-6.5%) or glycerol (2-7%) added to sperm extender with an osmolarity of 310 mOsm. Concentrations of 4.6% ethylene glycol and 5% glycerol resulted in higher degree of MCM sperm viability of 60.3% and 60.8% respectively. The results suggests that both cryoprotectants are suitable for MCM sperm freezing

3.  Hamilton-thorne IVOSII CASA system was used to characterize sperm kinetics and variations in sperm motility and progressive motility of sperm frozen with ethylene glycol compared with glycerol were not statistically different and the effect of freezing on other sperm kinetic characteristics was minimal and insignificant, however sperm frozen in glycerol had slightly higher VSL values (straight line velocity) which is considered a significant parameter in evaluating effects of freezing on sperm function.

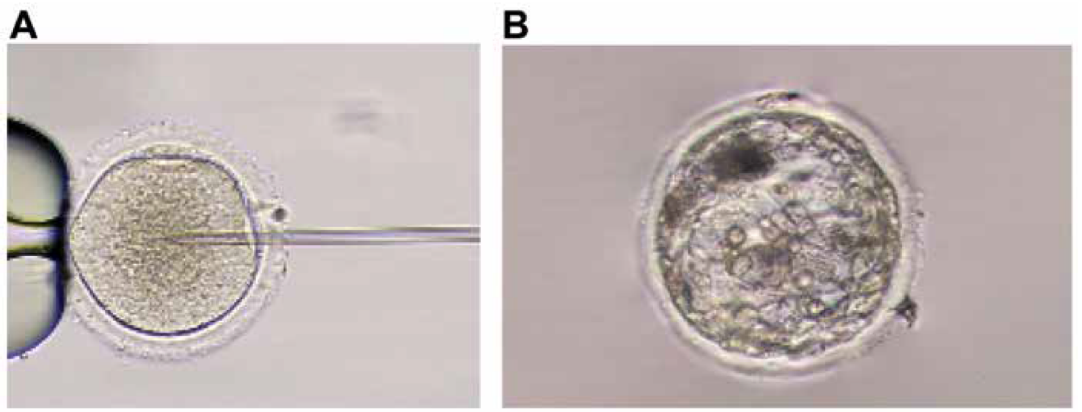


CASA IVOSII sperm movement tracking

* Red square - static , nonmotile
* Green track – motile sperm
* Yellow – late tracking of sperm
* Turquoise – progressive motile sperm

4.  Frozen thawed sperm samples were used to fertilize MCM oocytes by ICSI (intracytoplasmic sperm injection) to confirm that frozen thawed sperm retain their fertilization potential. Successful fertilization was achieved in 12 out of 15 oocytes (80%) as shown by formation of 3 pronuclei. This confirms that frozen MCM sperm retain fertilization capacity after freezing.

5.   MCM Sperm cryopreserved with glycerol was used for ICSI procedure as improved sperm motility is observed using this, and that ethylene glycol can potentially cause embryotoxicity



A.   MCM oocyte undergoing ICSI

B.  MCM blastocysts derived from oocyte fertilized by cryopreserved sperm

Conclusion: The study revealed that viability and motility parameters of MCM sperm frozen in defined conditions was similar to those sperm frozen with egg yolk containing extenders and much better than those of cynomolgus sperm frozen in commercial egg yolk-free medium. MCM sperm cryopreserved in chemically defined extender retained fertilization capacity in vitro.

QUESTIONS

1. Using egg yolk as an extender for freezing media can cause which of the following?

a.  Sperm agglutination

b.  No risk of contaminating sperm with viruses or pathogen

c.   Can be easily used for ICSI procedure

d.  Can easily maintain consistency in the freeing procedure

2.   T/F: Mauritian cynomolgus macaques have very limited MHC diversity consisting of only 10 common haplotypes.

ANSWERS

1.   a. Using egg yolk can cause sperm agglutination, risk of contaminating sperm with viruses/pathogens from egg yolk, complicates sperm manipulation, hard to maintain consistency in freezing procedure.

2.  False - Mauritian cynos have limited MHC diversity an only has “7” common haplotypes (M1-M7)

***Husbandry***

**Lovasz et al. Effects on Mouse Food Consumption After Exposure to Bedding from Sick Mice or Healthy Mice, pp. 687-694**

Domain 3: Research

Primary Species: Mouse(*Mus musculus*)

SUMMARY: Because mice are considered an empathetic species, it is possible that an "empathy state" exists for control mice that are housed in the same room as sick mice. At the institution where this study was conducted, it was noticed that control mice had decreased food intake, similar to mice with pancreatic ductal adenocarcinoma (PDAC) experiencing cachexia. In order to test the hypothesis that the decreased food intake was due to an olfactory mediated "empathy state," food intake, body weight, and food spillage over 48 hours were compared across naive male (n=20) and female (n=20) C57BL6/J mice exposed to either soiled bedding from mice with PDAC induced cachexia (sick bedding) or soiled bedding from control mice in the PDAC study (control bedding). Food intake measurements included the measurement of feed in the cage top and food granules within the cage, commonly referred to as "orts".

At 24 hours, female mice exposed to sick bedding had a statistically significant decrease in food intake, but not at 48 hours. At 48 hours, male mice exposed to sick bedding had a statistically significant decrease in food intake, but not at 24 hours. Female mice exposed to sick bedding had a statistically significant decrease in orts measured at 12 hours. Male mice exposed to sick bedding had a statistically significant increase in orts measured at 4 hours. Retrospective comparisons between PDAC and control mice in the absence or presence of an empathy state revealed a minimum necessary sample size of 16 mice if the empathy state did not exist and 28 mice if the empathy state did exist. The study pointed out that more mice would need to be studied to prove their hypothesis, but using more mice was contrary to the goal of using fewer animals. Also, the importance of using sex as a biologic variable is highlighted in this study, as evidenced by the differing data between the two sexes.

QUESTIONS

1. What term describes wasted pieces of food that are found in the bedding?

a. Grinders

b. Orts

c. Granules

d. Crumbs

2. T/F: PDAC mice increasingly grind and waste their food as their disease progresses, therefore weighing food in the hopper alone is inadequate for food intake studies.

ANSWERS

1. b

2. True

**Cantara et al. Comparative Effects of 1/4-inch and 1/8-inch Corncob Bedding on Cage Ammonia Levels, Behavior, and Respiratory Pathology of Male C57BL/6 and 129S1/Svlm Mice, pp. 695-702**

Domain 3

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Corncob is a standard bedding material for laboratory rodents, but no studies have compared how the 2 commercially available sizes of pellets (1/4 and 1/8 in.) might differ in their experimental and environmental effects. Ammonia is a substantial concern regarding the quality of the microenvironment, and the ability of corncob to mitigate ammonia has only been studied under a few conditions. The effects of bedding on ammonia levels in static cages, particularly those containing male mice, which produce more urine than female mice are not well known.

The purpose of this study was to compare the effects of 1/4 and 1/8 in. corncob bedding on behavior, intracage ammonia levels, and pathology of the respiratory and ocular tissues of mice. Male mice in static cages were used to better determine the effects of bedding under conditions likely to be associated with relatively high ammonia levels. They also used male mice because behavioral research is heavily biased toward male mice to avoid issues related to the estrous cycle. The mouse stains (C57BL/6 and 129S1/Svlm) were chosen because they are commonly used background strains in behavioral research. The hypothesis was that the pellet size of corncob bedding would not significantly alter effects on the measured parameters. 60 male mice (C57BL/6 and 129S1/Svlm) were housed in static, filter-top cages containing 1 of the 2 bedding types for the duration of the study (12 wk). 12 naïve mice (6 of each strains) served as negative controls for histopathology.

Intracage ammonia levels were measured on days 0, 3, 5, and 7 during week 7 of the study. Overall, bedding size did not have a significant effect on ammonia levels. Ammonia levels did not differ significantly between mouse strains but increased significantly over time in all cages for both bedding sizes, supporting the hypothesis that ammonia levels would not differ between the 2 bedding sizes because although pellet size differed, the bedding material is the same.

Histopathology of respiratory and ocular tissues was used to evaluate potential adverse effects associated with the bedding. The lesions in experimental mice were limited to mild inflammation in the rostral nasal cavity; these lesions were significantly different from controls but were not affected by bedding or strain. No animals exhibited clinical signs of illness during the study.

Behavior was evaluated by using circadian rhythm, open field, and Morris water-maze tests. Bedding size did not affect the behavioral tests, whereas strain did have an effect. As expected, all animals were initially very active when placed in the circadian rhythm caging because it was a novel environment. As previously reported, B6 mice were more active than 129 mice in the circadian rhythm test.

This study shows that although the pellet size of corncob bedding is unlikely to affect ammonia levels and respiratory pathology, intracage ammonia levels can be higher than those measured in static cages of most previous studies. In conclusion, 1/4-in. and 1/8-in. corncob beddings have comparable effects on cage ammonia levels and the behavior and respiratory pathology in male mice of the strains tested.

QUESTIONS (True or False)

1. Exposure to ammonia in rodents can result in inflammation, necrosis, or proliferation of epithelial tissues.

2. Bedding material has been shown to affect exploratory and spontaneous behavior in male mice

3. Male mice excrete as much as twice as much urine as female mice

ANSWERS

1. True: In addition, corneal ulcers and other ocular lesions have been associated with ammonia exposure.

2. True

3. True

***Management***

**Kimura et al. Examination of Material Compatibilities with Ionized and Vaporized Hydrogen Peroxide Decontamination, pp. 703-711**

Domain 4: Animal Care; Task 3: Management of laboratory animal facility; Knowledge 3: Methods of sterilization, sanitation, and decontamination

SUMMARY:  Hydrogen Peroxide (HP) decontamination recently replaced ethylene oxide, because of its toxicity to personnel, and formaldehyde which has been classified as a human carcinogen. HP is an oxidizing agent which produces free radicals that damage microbial DNA and cell constituents. Gaseous HP is a broad spectrum antimicrobial with virucidal, fungicidal, and sporicidal activities and prion inactivation.

Ionized hydrogen peroxide (iHP) decontamination system consists of 3 components: a generator, a spray pod, and a scrubber. The technology uses the processes of ionization and nucleation to create small electrically charged droplets that bond to microorganisms on material surfaces or in the environment. The procedure consists of gassing, dwell time and aeration. Gassing starts with a 7.5% HP liquid solution, passed under pressure through a nozzle device which creates a fine mist. The mist droplets pass through the cold arc between 2 high energy electrodes at 17,000V. During the dwell time, the charged aerosol droplets stay suspended in the air and are uniformly dispersed on the material surface. During aeration, residual iHP is catalytically decomposed into water and oxygen by recirculation through the scrubber or ventilation system.

During vaporized hydrogen peroxide (VHP) decontamination, a mobile generator converts 35% concentrated liquid HP to VHP. The standard VHP cycle consists of 4 phases: dehumidification, conditioning, decontamination, and aeration. During dehumidification the relative humidity is reduced by circulating the air in a closed loop. During conditioning, VHP was produced by vaporization of the 35% liquid HP. The decontamination phase ensures that peak VHP decontaminating concentrations of 800 to 1,000 ppm are maintained for 30 min. In the aeration phase, VHP is no longer introduced and any of the residual vapors are catalytically decomposed into water and oxygen by recirculation through the scrubber or ventilation system.

In this study 24 kinds of materials were exposed to 100 cycles of iHP in a test chamber. 36 kinds of materials were exposed to 200 cycles of VHP contamination in the test chamber. The VHP process was performed under dry and condensation conditions where the test chamber did not undergo dehumidification.

Slight changes were found in the die-cast brass and zinc immediately after exposure to iHP decontamination. Chromium plating G and S, copper and copper screws underwent changes. Bronze plating, chromate-treated steel and stainless steel began to show serious degradation after 70-90 cycles. Repeated iHP decontamination caused marked damage in epoxy, silicone, and urethane coating materials. The urethane coating started to show damage after 70 cycles showing blisters on the surface. High concentrations of iHP associated with condensation changes caused severe changes in the materials after repeated exposure.

Few changes in gross appearance of resins occurred throughout the dry process of VHP decontamination. Several resins started to lose their plasticity, while nylon began to soften by 10 cycles. Polysulfide, acrylic, and urethane caulking compound showed moderate to severe damage, whereas silicone caulking compound remained unchanged. Bleaching was observed on wooden dowels. While damage to galvanized sheet iron, nickel plated steel and unichrome plating was slight, there was blister formation in the color coated sheet iron. There was also early discoloration in the color coating aluminum materials and slight corrosion of copper, brass, and steel.

During VHP with condensation conditions, HP droplets were found on the surface of the materials. Condensation of VHP had adverse effects on polytetrafluoroethylene, butyl rubber, nitrile butyl rubber, and polyethylene. Nylon 66 and natural rubber softened, and acrylic materials showed a large number of very fine chemical cracks after about 100 cycles. There was severe damage to polysulfide, acrylic and urethane caulking compound. Changes were seen on wooden dowels and severe corrosion of galvanized sheet iron, color coated sheet iron, nickel plated steel, unichrome plating steel, copper and brass. VHP liquefied under humid conditions leaving high concentrations of HP in droplet form that would have been otherwise be compatible.

Findings show that, while both iHP and VHP unavoidably contributed to the development of chemical and physical changes to many materials tested, the dehumidification process was essential to minimize damage to materials. VHP decontamination should preferably be performed under conditions of less than 30% relative humidity.

QUESTIONS

1. T/F: Ethylene oxide and formaldehyde are safe alternatives to hydrogen peroxide decontamination.

2. T/F: The amount of corrosion and color changes were equal during VHP decontamination under wet and dry conditions.

3. In order to reduce material corrosion, VHP decontamination is preferably performed under conditions of :

a. High relative humidity

b. Low relative humidity

c. High temperature

d. Low temperature

4. T/F: Only VHP causes development of chemical and physical changes during decontamination while iHP does not.

5. The standard VHP cycle consists of 4 phases:

a. Conditioning, dehumidification, decontamination, and aeration

b. Dehumidification, conditioning, decontamination, and aeration

c. Decontamination, dehumidification, conditioning and aeration

6. The standard iHP procedure consists of:

a. Aeration, gassing, dwell time

b. Dwell time, gassing, and aeration

c. Gassing, dwell time and aeration

7. During aeration, residual HP is catalytically decomposed into water and oxygen by recirculation through the scrubber or ventilation system. (T/F)

8. Gaseous HP is a broad spectrum antimicrobial with:

a. Virucidal, fungicidal, and sporicidal activities but not prion inactivation

b. Virucidal, fungicidal, and sporicidal activities and prion inactivation.

c. Virucidal and fungicidal activities  but not sporicidal or prion inactivation

ANSWERS

1. F

2. F

3. b

4. F

5. b

6. c

7. T

8. b

**Pearson et al. Metaphylactic Antibiotic Treatment to Prevent the Transmission of *Corynebacterium bovis* to Immunocompromised Mouse Offspring, pp. 712-718**

**Domain 1, T1 and 2, K4**

**SUMMARY:** C. bovis is a common bacterial pathogen among immunocompromised mouse colonies. In athymic nude mice clinical signs include: an asymptomatic persistent carrier state and 2 to 7 days of dermal hyperkeratosis, dehydration, lethargy, and decreased body condition. In haired immunocompromised mouse strains i.e. NSG mice clinical signs include: rough hair coat, decreased body condition, scaly skin, alopecia, conjunctivitis, and erythematous pinnae. This group investigated a novel method to produce immunocompromised offspring free of C. bovis from infected NSG breeders.

Previous studies have shown that antibiotic administration can prevent detection of C. bovis from infected mouse skin by culture (during treatment). Subsequently the animals were found to be positive via culture after discontinuing treatment with definitive clearance of the pathogen in only 13% of infected adult mice. The goal of this study was to investigate metaphylactic use of amoxicillin-clavulanic acid for C. bovis infected NSG breeding pairs in order to prevent post-partum transmission of the pathogen prior to weaning. Their hypothesis was that metaphylactic antibiotics would either eliminate all viable C. bovis or decrease skin burden to undetectable levels in adult breeders. This would facilitate a C. bovis free window during which transmission to neonates and weanlings would be prevented. This would create a noninvasive method to eliminate C. bovis from unique immunocompromised strains.

The group was able to demonstrate that metaphylactic administration of amoxicillin-clavulanic acid decreased C. bovis skin copy numbers based on qPCR analysis and animals were determined to be culture negative while on antibiotics. This treatment along with weekly cage changes during gestation and changing gloves prior to handling anything within the cage, they were able to prevent transmission of infection to neonates and weanlings. The group did note that pup mortality for the antibiotic cohorts occurred in 50% and 60% of the litters respectively. It was hypothesized that the increased cage-change out frequency after pairing and the complete removal and replacement of nesting material might have contributed to the poor litter survivability in the antibiotic treated group.

**QUESTIONS**

1.  What is metaphylaxis?

2.  Describe the clinical signs and lesions of C. bovis.

3.  Microscopically what lesions are present?

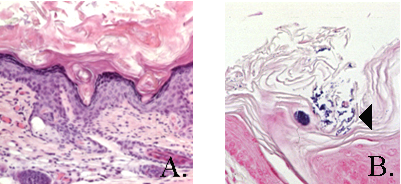
**ANSWERS**

1.  The treatment of a group of animals after the diagnosis of infection and/or clinical disease in part of the group, with the aim of preventing the spread of infectious disease to animals in close contact and at considerable risk and which may already be (sub-clinically) infected or incubating the disease.

2.  Clinical signs associated with C. bovis infection in nude or SCID mice include a wide-spread scaly dermatitis, hence the name “scaly skin disease”. In nude mice, the signs are most commonly seen on the dorsum, and in SCID mice, the scaling is accompanied by alopecic areas. Microscopically, skin sections reveal epidermal hyperplasia (acanthosis) and an orthokeratotic hyperkeratosis. Macrophages and neutrophils infiltrate the dermis.

C. bovis rapidly causes clinical signs in naïve animals. Lesions that appear 7-10 days after exposure to infection (for example, exposure to infected animals or a contaminated facility) are consistent with, and characteristic of, a new C. bovis infection. If animals survive the infection (most do), the hyperkeratosis disappears, but the acanthosis remains, as does a slight increase in inflammatory cells in the dermis. Animals remain infected after clinical signs have resolved and continue to shed organisms. Animals from a colony that is enzootically infected with C. bovis are persistently infected, but may show signs less frequently than would naïve animals.

3.  Skin lesions consist of prominent [acanthosis](https://www.sciencedirect.com/topics/medicine-and-dentistry/acanthosis) and moderate hyperkeratosis accompanied by mild nonsuppurative inflammation.



***Anesthesia***

**Klune et al. Hypothermia During General Anesthesia Interferes with Pain Assessment in Laboratory Rats (*Rattus norvegicus*), pp. 719-725**

Domain 1

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Timely assessment of ongoing pain is required to maintain laboratory animal welfare. The objective of this study was to determine if hypothermia induced by general anesthesia confounds postanesthetic pain assessment of rats with the Rat Grimace Scale (RGS). The RGS assesses changes in orbital tightening, nose/cheek flattening, and ear and whisker position changes to identify pain. Twenty 10-13wk old Sprague Dawley male and female rats were divided into two groups: warmed with a heating pad and unwarmed on a thin synthetic sheet. Both groups underwent anesthesia with Isoflurane for 30 minutes. Normothermia was successfully maintained in warmed rats under anesthesia whereas unwarmed rats became hypothermic beginning 15 minutes after induction.  After return of sternal recumbency rectal temperatures and RGS data were collected. Scores of all the RGS actions were averaged for each observation over a 10 minute observation period. RGS scores of unwarmed rats were significantly higher than baseline at all timepoints after anesthesia. In contrast, warmed rats had slightly higher RGS scores from baseline at 5 and 120 minutes only and from 60 min after recovery. Unwarmed rats had higher RGS scores at 8 and 180 minutes and took longer to attain sternal recumbency after anesthesia. There were no gender difference between male and female rats. Overall, this study showed that hypothermia induced by isoflurane anesthesia increases RGS scores.

QUESTIONS

1. Which of the following is **not** assessed by the Rat Grimace Scale

a. Orbital tightening

b. Ear positioning

c. Hunched appearance

d. Whisker positioning

2. T/F: Hypothermia induced by anesthesia does not present a confounding factor for accurate RGS scoring

3. T/F: The results of this study did not differ between male and female rats

ANSWERS

1. c

2. False-This study showed hypothermia **does** present a confounding factor

3. True

**Stewart et al. Injection-site Reactions to Sustained-released Meloxicam in Sprague-Dawley Rats, pp. 726-731**

Domain 3: Research

Primary Species: Mouse (*Mus musculus)*

SUMMARY: Sustained-release formulations of Meloxicam (MSR) are optimal for maintaining analgesia while reducing animal handling stress and personnel labor. MRS efficacy throughout the full 72-h window has been demonstrated to various degrees; however, adverse reactions have not specifically been assessed. Injection site lesions of variety severity have been noted in MSR studies in different species, but it has not been identified in rodents. The current study determined the rate of localized tissue reactions after a single subcutaneous injection of MSR in rats; plus, characterized skin lesions developed at 7 days and 14 days post injection through histopathology.

Sprague-Dawley female and male rats were randomly assigned to one of two treatment groups, MSR (n=16) or sterile saline control (SC, n=6). Each rat received a single subcutaneous injection of MSR or equivalent volume of sterile saline; and injections were administered over the sacral region. Then, rats were examined daily and palpated for lesions at or near the injection site for 7 or 14 days after injection by a single treatment-blinded observer.  All palpated masses received a score for mass and erythema that characterize the presentation and severity of the lesion.

All rats in the MSR group presented mass lesion after injection, whereas none of the rats in the SC group developed masses or erythemic lesions; and there was no difference in the prevalence of mass or erythema between males and female rats. Plus, the median time to first mass lesion in the MSR group was 3 days; the trajectories of mass lesion severity in this group showed a rapid progression from stage 1 at onset at day 2 or 3, to stage 2 for almost all animals by day 5 or 6. Four out of the sixteen rats in the MSR group presented erythema lesions, of the 4 rats to develop erythema lesions, 3 were scored at level 3 with severe lesions and 2 had draining tracts (ulcerations). Histologically, at 7 day post MSR injection, the subcutaneous nodules looked as foci necrotizing panniculitis with intralesional amphophilic material and extensive peripheral fibroplasia. At day 14 post MSR injection, the inflammatory response showed less necrosis and more foamy macrophages interpreted as granulomatous to xanthomatous panniculitis.

Although MSR analgesic offers many benefits, it is recommended to evaluate the possibility of lesion formation, compromised animal welfare, and potential confounding study factors when planning a study.

QUESTIONS

1. T/F: The median presentation times of lesions post MSR injection were 2 to 3 days for palpable masses, 5 to 6 days for the mass to develop clearly defined borders, and more than 7 days for erythema to develop.

2.  Which of the following statements should be considered when planning to use MRS in rats?

a. Establishing the safety of MSR in other strains of rats before using it in research studies, especially in where inflammatory responses may confound study outcomes.

b.  Palpation of the injection sites in addition to careful observation of animals that receive MSR.

c.  MSR tolerance could be assessed in a subset of animals before administering it to study subjects.

d.  All of the above.

ANSWERS

1.  True

2.  d

**Musk et al. A Comparison of Buffered Tricaine Methanesulfonate (MS-222) and Isoeugenol Anesthesia for Caudal Fin Clipping in Zebrafish (*Danio rerio*), pp. 732-736**

Domain 3: Research

Primary Species: Zebrafish (*Danio rerio*)

SUMMARY: Finding anesthetic drugs that are both efficacious and safe to use is difficult, particularly in zebrafish, as there are few drugs to choose from, few methods of administration, and limited capacity to monitor animals under anesthesia. Nevertheless, anesthesia mitigates pain and suffering and may be beneficial during the performance of invasive technical procedures such as caudal fin clipping of zebrafish. The purpose of this study was to compare the safety and efficacy of single doses of buffered tricaine Methanesulfonate (MS-222) and isoeugenol for zebrafish undergoing anesthesia for caudal fin clipping. The hypothesis were that induction of anesthesia and recovery would be longer with isoeugenol than with MS-222 and the safety of the two drugs would be comparable as assessed by morbidity and mortality.

Eighty zebrafish (AB strain) at 9mo of age were allocated two one of two groups: buffered MS-222 (n=40) or isoeugenol (n=40). One fish was selected at a time and placed in an anesthetic bath (500mL). Fish were observed until stage 5 anesthesia was apparent and the time was recorded. Clipping of the caudal fin was performed in a culture dish with a scalpel blade. The fish was then placed in a recovery tank and observed until swimming normally. Post procedural monitoring was performed at 1,2,3,4, and 24 hours after the procedure. Observations included attributing a score 0,1, or 2 (0 =normal, 1 = slightly or intermittently abnormal, 2 = moderately of consistently abnormal) to 8 parameters: physical appearance, food consumption, respiratory pattern, swimming behavior, wound site, and any other presenting signs. The morning after the procedure, fish were euthanized by in an ice slurry and weighed. Following euthanasia sex was confirmed and 18 males and 22 females were exposed to buffered MS-222, and 21 males and 19 females were exposed to isoeugenol.

The time to reach stage 5 anesthesia was shorter in the isoeugenol group than in the MS-222 group. Time to recovery from anesthesia was shorter in the MS-222 group compared to the isoeugenol group. Male and female fish did not differ with time to stage 5 anesthesia or recovery time. No apparent displays of distress or aversion to anesthesia and no movement or response to the procedure was observed in either group. There was no difference in the number of fish that reached stage 5 anesthesia with either drug. One male fish in the buffered MS-222 group was found dead at the 1-hour post – procedural monitoring time point, however, there was no difference between groups in the proportion of fish that survived anesthesia to the end of the experiment. Otherwise, post procedural monitoring scores were 0 for 79 zebrafish.

The study concluded that the safety and efficacy of buffered MS-222 and isoeugenol for adult zebrafish undergoing anesthesia for caudal fin clipping was similar, suggesting that while pharmacokinetics of isoeugenol and MS-222 differ, the two compounds are not significantly different in efficacy at the dosage given.

QUESTIONS

1. Isoeugenol is a compound similar to eugenol and may also cause all of the following except for\_\_\_\_\_\_\_\_\_\_.
   1. Inhibit sodium channels
   2. Inhibit NMDA receptors
   3. Potentiate GABAAreceptors
   4. Stimulate calcium channels
   5. Inhibit potassium channel
2. Stage 2 anesthesia is characterized by:
   1. Motion and breathing reduced
   2. Partial loss of equilibrium reactive to touch stimuli
   3. Total loss of equilibrium, no reaction to touch stimuli
   4. Breathing and heartbeat stop overdose - eventual death

ANSWERS

1. d
2. b

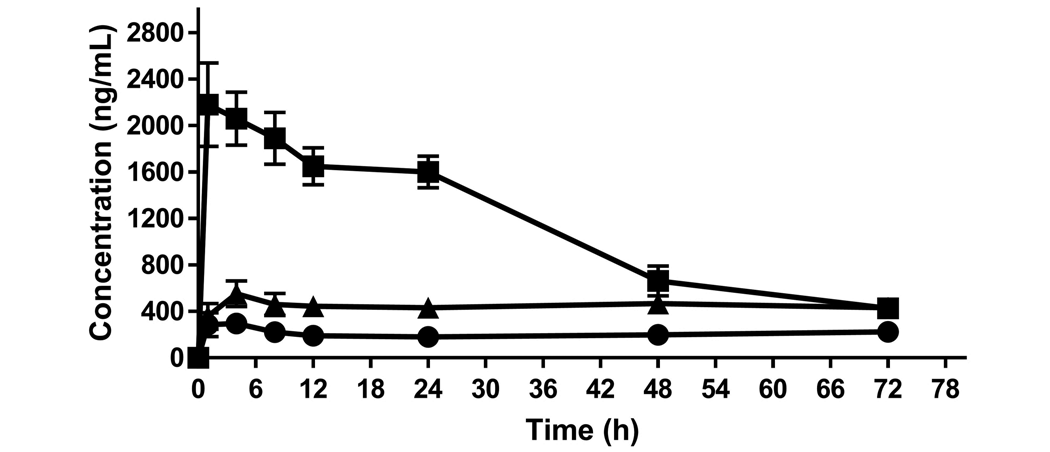
**Smith et al. Pharmacokinetics of Sustained-release, Oral, and Subcutaneous Meloxicam over 72 Hours in Male Beagle Dogs, pp. 737-741**

Domain 2: Management of Pain and Distress

Primary Species: Dog (*Canis familiaris*)

**SUMMARY:** Meloxicam is a commonly used COX-2 inhibitor with long half-life and limited GI side effects. Meloxicam inhibits prostaglandin synthesis which has anti-inflammatory effects and provides pain relief. Traditional formulations have a half-life in dogs just below 24 hours. Sustained release meloxicam (Melox-SR) has exceeded therapeutic levels in cynos for 48-72 hours. This study evaluated 3 formulations: oral, standard SQ and sustained release meloxicam. The purpose was to determine if the sustained release formulation achieved longer therapeutic concentration vs the standard formulations.

Results: Meloxicam concentration was determined over 72 hours in the three groups. Baseline samples showed no meloxicam present after a washout period. Sustained release meloxicam was given once SQ, while the oral and standard SQ formulations were given SID. The sustained release concentration peaked at 1 hour after administration, whereas the PO and standard SQ peaked at much lower concentrations at 4 hours. Sustained release had significantly higher concentrations than standard SQ until 48 hours and the oral form until 72 hours. The standard SQ had significantly higher concentrations than the oral form through the entire 72-hour period. No injection site lesions were noted. One dog developed signs of intestinal hemorrhage after injection but was diagnosed with small intestinal leiomyosarcoma at necropsy. CBC and chemistry values after sustained release meloxicam did not differ from baseline. The half-life of the standard SQ and oral forms was not determined due to the few collection time points. The minimum effective concentration of meloxicam in humans to inhibit COX1 & COX2 is 2000ng/mL & 80ng/mL, respectively. Applying these same values to dogs means that dogs may be at risk of GI ulceration when given the sustained release meloxicam formulation as the max concentration exceeded 2000ng/mL.



**Figure 1.** Plasma concentration (mean ± 1 SD [error bars]) of meloxicam after dosing with Melox-SR (n = 6, squares), Melox-SC (n = 5, triangles), and Melox-PO (n = 6, circles) in adult male beagles. Melox-PO and Melox-SC samples were collected prior to subsequent drug administrations at the 24- and 48-h time points. Plasma concentrations at these time points represent the nadirs.

**QUESTIONS**

1.  Meloxicam is a selective inhibitor of \_\_\_\_\_\_?

a.  COX1

b.  COX2

2.  T/F: Ketoprofen is a nonselective NSAID and inhibits both COX1 and COX2.

3.   T/F: Cyclooxygenase 2 is constitutively expressed and is important for the maintenance of the GI mucosa.

**ANSWERS**

1. b

2. True

3. False

***Euthanasia***

**Nichols et al. Cardiovascular and Metabolic Responses to Carbon Dioxide Euthanasia in Conscious and Anesthetized Rats, pp. 742-749**

Domain 3: Research

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY:CO2 euthanasia has been (and continues to be) under scrutiny in the laboratory animal field due to its potential to be aversive, painful to the mucus membranes, and cause distress prior to loss of consciousness (LOC). Upon exposure, LOC occurs due to respiratory acidosis, and death via asphyxiation. In 2020, the AVMA amended its recommendations from a displacement rate of 10-30% to 30-70%. CO2 is otherwise relatively safe to the human operator, inexpensive and easily accessible. Isoflurane anesthesia prior to CO2 euthanasia has been proposed to mitigate the negative effects of CO2 prior to LOC.

The aim of this study was to compare the physiological and behavioral responses of rats implanted with telemetry devices euthanized by CO2 only (C) or CO2 following pre-anesthesia with isoflurane (IC). Male Sprague-Dawley rats were surgically implanted with telemetry devices to measure mean arterial blood pressure (MAP), ECG, and plasma glucose (BG) and allowed to fully recover (~2 weeks). Baseline measurements were collected for a 4-hour period, 24 hours prior to the planned euthanasia event. All animals remained in their home cages for euthanasia but were moved from their housing room into a separate room. For C, the CO2 displacement rate used was 30% of the volume chamber per minute with 100% CO2. For IC, animals were first anesthetized with 5% isoflurane until LOC and immediately moved into a chamber pre-filled with 100% CO2. Video footage was taken for behavioral analysis. Immediately after euthanasia, blood from the tail vein was taken for a final glucose measurement.

For both groups, HR and MAP increased when the modified cage lids were placed on the home cage, perhaps indicating a mild, acute stress response. Once CO2 delivery was initiated for C, MAP was (not significantly) increased through LOC. Time to LOC and death was significantly faster in C. Although not significant, 4/6 IC rats showed aversive behaviors upon exposure to isoflurane versus 1/7 C rats upon exposure to CO2. BG was stable for both groups except for a significant, short drop when C rats were initially exposed to CO2. The authors suggest that neither methods cause clear distress prior to LOC, but conscious animals being exposed directly to CO2 for euthanasia may overall be more humane. They recommend smooth transport and appropriate time to acclimate to the new macroenvironment prior to euthanasia, when possible.

QUESTIONS

1. Hypercapnia is known to (increase/decrease) blood pressure through rapid release of (what hormone(s))?
2. Which receptor is isoflurane proposed to act upon to induce anesthesia?

ANSWERS

1. Increase, angiotensin II and vasopressin
2. GABAA Receptor