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**ORIGINAL RESEARCH**

***Husbandry***

**Reiter et al. Effect of Home Cage Bedding in the Induction Chamber on Serum Cortisol and Corticosterone Levels in Response to Isoflurane-induced Anesthesia in C57BL/6J Mice, pp. 118-121**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The authors goal was to develop refinement techniques to decrease the level of stress that mice may experience when anesthetized in induction chamber. Mice are routinely anesthetized with isoflurane in an induction chamber. The AVMA Guidelines for the Euthanasia of Animals states that distress should be minimized during euthanasia but does not address this point regarding induction of anesthesia. The authors evaluated the potential for familiar surroundings to reduce the adrenocortical response of mice during anesthesia induction with isoflurane. However, the authors found that adding bedding from the animals' home cage to the induction chamber failed to significantly reduce serum cortisol or corticosterone levels in male and female C57BL/6J mice. These results indicate that familiar surroundings do not appear sufficient to reduce the adrenocortical response of mice during anesthesia induction with isoflurane.

QUESTIONS

1. What hormone is used as index for stress activation in mice?

a. Cortisol

b. Corticosterone

c. Estrogen

d. Both a & b

2. What organ release hormones responsible for stress activation in mice?

a. Adrenal gland

b. Kidneys

c. Pituitary gland

d. All of the above

ANSWERS

1. d

2. a

**Theil et al. Effects of Human Management Events on Conspecific Aggression in Captive Rhesus Macaques (*Macaca mulatta*), pp. 122-130**

Domain 1: Prevention of spontaneous/unintended disease or condition (injury)

Domain 4: Animal Care (husbandry)

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Conspecific aggression at primate research facilities can result in trauma and influence animal wellbeing and confound research studies. While aggression is part of normal social interactions, the entry of human caretakers/researchers into the environment and interactions between people and animals was determined to increase the frequency of aggression events. Over 18 months, 7 social groups in large outdoor cages were observed for agonistic encounters, and these observations compared against a log of human activities (feeding, cleaning/maintenance, animal catch and release actions, approach without entry). Statistical analysis was applied and a significant increase in aggression frequency was noted in the 30 minutes after completion of a task compared with the time prior to entry and during the task. An increase in aggression was also associated with increased numbers of events in a day (2+ compared to 0 or 1). A specific individual technician was noted to have a more profound effect compared to other technicians. Human management events were concluded to impact aggression frequency in the social groups; attention should be given to this impact when planning management approaches to minimize trauma instances.

QUESTIONS (True or False)

1. Aggression in group-housed macaques is observed to increase following husbandry management activities.

2. Rhesus macaques live in social groupings of less than 10 individuals.

3. Aggressive behaviors to establish and reinforce social hierarchies are part of normal social interaction in macaques.

ANSWERS

1. True

2. False. Typical troops number between 20 and 200 individuals in nature.

3. True

***Animal Health Surveillance***

**Meritet et al. Development of Quantitative Real-Time PCR Assays for Postmortem Detection of *Mycobacterium* spp. Common in Zebrafish (*Danio rerio*) Research Colonies, pp. 131-141**

Domain 1; Task 3

Secondary Species: Zebrafish (*Danio rerio*)

SUMMARY: Zebrafish (*Danio rerio*) are a popular animal model. Diagnostic and screening tests are necessary to remove confounding comorbidities and loss of zebrafish to mycobacterioses. The three spp. of most concern are *Mycobacterium marinum*, *M. haemophilum*, and *M. chelonae*. Mycobacterial infections in experimental animals also pose a health threat to laboratory personnel.

Molecular diagnostic methods are the most practical and efficient approach to detect and identify mycobacteria to the species level, because many are difficult to grow in culture and because biochemical tests are often not informative.  These PCR methods may involve amplification of DNA from fresh, frozen, or ethanol-preserved tissues and target areas of high discriminatory power, such as hsp65, rpoB, and the 16S– 23S internal transcriber spacer.

Alternatively, species and strain identifications can be rapidly and accurately obtained by using PCR tests of bacteria isolated in culture. In zebrafish, PCR testing for mycobacteria by using formalin-fixed or paraffin-embedded (or both) material is particularly useful, because histopathology is generally the primary diagnostic method used for this species, and fresh tissues and cultures are often unavailable.

The current study developed simplex (that is, using a single primer or probe set to detect a particular species), real-time quantitative PCR (qPCR) assays for each of the aforementioned mycobacterial species for 3 types of samples: bacterial isolates, fresh-frozen fish, and formalin-fixed paraffin-embedded (FFPE) fish. The assays target heatshock protein 65 gene of the three species, and are both highly specific and sensitive for fresh-frozen samples and highly specific and moderately sensitive for formalin-fixed paraffin-embedded (FFPE) samples. The group infected fish experimentally.  Fish were anesthetized with buffered MS222 and injected with the bacteria.

Two sampling techniques for FFPE samples of sagittally sectioned zebrafish were evaluated. Both paraffin cores targeting granulomas containing bacteria and scrolls from the entire fish yielded DNA of equivalent quantity and purity. The diagnostic sensitivity of cores was superior to that of scrolls for *M. chelonae* and *M. haemophilum* but not *M. marinum*. The assays are cost-effective and ideally suited to diagnosing common Mycobacterium spp. infections in zebrafish.

Primers were designed based on published sequences of the bacterial heat-shock protein 65 gene, which is highly conserved within the Mycobacterium genus but variable enough to distinguish between Mycobacterium species.  Control isolates were: attenuated BCG Pasteur and K10 *M. avium* subsp. Paratuberculosis, Streptococcus inia, Nocardia nova, Pasteurella multocida, and Corynebacterium pseudotuberculosis isolated from diagnostic samples and phenotypically characterized. Adult wild-type zebrafish of both sexes were acquired from facility that is SPF for Pseudoloma neurophilia and has a very low background infection with M. chelonae. Primer and probe sets were analyzed for sequence similarity to other Mycobacterium species and were used in a BLAST search to identify sequence similarity across all bacterial genera. Each primer and probe set was examined for sequence similarity with the other sets.

Diagnostic specificity and sensitivity were calculated using the current ‘gold standard’ of diagnosis: detection of acid-fast bacteria in granulomas in situ. Findings from microscopic examination of tissue samples stained with hematoxylin and eosin or acid-fast stain. Acid-fast bacteria were present in the spleen of all zebrafish. Other common body sites were kidney, liver, and ovaries and within or adjacent to the swim bladder. Infection with M. chelonae presented in about half of the fish with large granulomas in the ovary or adjacent to the swim bladder; in the other half, acid-fast bacteria lined the lumen of the swim bladder. M. haemophilum-infected zebrafish had large granulomas, which were in the kidney in all fish as well as (in decreasing prevalence) adjacent to the swim bladder, in the liver, or in the pericardium.

Mycobacteria, in general, are known to have a high limit of detection in PCR assays (that is, require more organisms to be detected), given that robust cell walls protect their DNA. Even with a lysozyme step added prior to nucleic acid isolation, it is difficult to obtain a large quantity of DNA. The number of colony-forming units required to detect mycobacteria by using PCR assays is greater than those documented for other bacteria.

QUESTIONS

1. Which of the following mycobacterium species causes subclinical infection with extensive internal and high bacterial load?

a. *M. marinum*

b.  *M. haemophilum*

c.  *M. chelonae*

d.   *M. fortuitum*

2. Mycobacterium can cause which of the following clinical signs in laboratory personnel working with infected fish?

a.  Rapidly developing cutaneous lesions that are not contagious

b.  Slowly developing cutaneous lesions that are not contagious

c.  Rapidly developing cutaneous lesions that are contagious

d.  Slowly developing cutaneous lesions that are contagious

3.  Choose the correct answers to the blanks: Due to their cell wall composition, Mycobacterium have a \_\_\_\_\_\_\_\_limit of detection in PCR assays, meaning they require \_\_\_\_\_\_\_\_\_ organisms in order to be detected.

a. Low; more

b.  High; more

c. Low; less

d.  High; less

4.  Histological detection of mycobacteria requires:

a.  Acid fast stain

b.  Warthin-Starry stain

c. Gram stain

d.  Masson’s trichrome stain

ANSWERS

1. c

2. b

3. b

4. a

***Anesthesia***

**Janssen et al. Comparison of Atipamezole with Yohimbine for Antagonism of Xylazine in Mice Anesthetized with Ketamine and Xylazine, pp. 142-147**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY

Aim: Ketamine/Xylazine is a mainstay of rodent anesthesia and currently reversal of Xylazine sedation/ anesthesia is not practiced routinely in rodents. One of the reason could be the reluctance of researcher to introduce variables. In the present study, authors have argued that reversal will be beneficial for researchers and for the mice, as it will reduce the anesthetic recovery time without forcing investigators to change the anesthetic regimen. The study aimed to compare the 2 α2antagonists yohimbine and atipamezole and saline (as a control) to determine which is more clinically effective when injected IP for reversing the sedative effects of xylazine in male C57BL\6J mice anesthetized with ketamine and xylazine.

Study Design: In this crossover study using Latin squares randomization, 18 (C57BL/6J male mice, 5 weeks of age) mice were randomly assigned into groups of 3 and each group of mice was anesthetized on 3 occasions by using the same anesthetic cocktail. Animals were subjected to Xylazine (10mg/kg) and Ketamine (80mg/kg) anesthesia. Anesthesia was reversed with atipamizole (1mg/kg) or yohimbine (1.5mg/kg) or saline after 15 min of Xylazine/ Ketamine administration. All drugs diluted for delivery at 0.01mL/gm. All drugs administered by IP injection. Time (in minutes) from injection of the reversal agent until “return of righting reflex” was the primary dependent variable. Return of righting reflex was defined as ability to roll from dorsal to sternal recumbency 3 times within a 60 second period. Heart rate was logged every 5sec. using pulse ox (Kent Scientific) with data output to laptop.

Findings/Results:Atipamezole (mean ± 1 SD, 10.3 ± 6.5 min) resulted in the most rapid recovery of the mice to sternal recumbency, allowing for prompt return of the mice to the home cage after anesthesia, compared with yohimbine (21.3 ± 5.6 min) and saline (38.2 ± 7.5 min). The heart rate of mice given atipamezole (1 mg/kg IP; top graph) begins to rise almost immediately and is normal or mildly tachycardic within 4 min; some mice begin to recover the righting reflex shortly thereafter. Yohimbine (1.5 mg/kg IP, middle graph) causes a similar rise in heart rate as that of atipamezole, but there is a delay of 7 to 10 min before the first effect is seen. These data indicated that recovery time after intraperitoneal administration of atipamezole (1 mg/kg) is significantly faster than that after yohimbine (1.5 mg/kg IP), which is still faster than the time to recovery after no antagonist.

Conclusions: Results from this study concluded that If “Better” is defined as easier injection site, faster absorption, faster return of righting reflex, then Atipamizole can be recommended to investigators as “Better” than Yohimbine or spontaneous recovery without reversal. Recommendation to researchers to use atipamizole to recover mice that are currently not being reversed, or are being reversed with Yohimbine will have two results.

1) Reduction of many minutes of anesthesia per mouse, with associated physiologic benefits to perfusion, thermoregulation etc.

2) Cumulative reduction of man-hours of IACUC mandated observation of anesthetized mice by technicians and post-docs.

QUESTIONS

1.  Atipamizole is FDA approved for the reversal of Xylazine?      True/False

2. Atipamizole is marketed and FDA approved for medetomidine and dexmedetomidine? True/False

3. Which is not a α2 agonist

a. Xylazine

b. Detomidine

c. Medetomidine

d. Tolazoline

4. Name 3 α2 antagonists

5.  What is the atipamizole’s α2:α1 affinity ratio?

a. 40

b. 4826

c. 4000

d. 8526

6.  Atipamizole’s affinity for the α2 receptor is 100 times greater than that of Yohimbine? True/False

ANSWERS

1. False

2.  True

3.  d

4.  Yohimbine, Atipamizole, Tolazoline

5.  d (Yohimbine has 40)

6.  True

**Wilding et al. Benefits of 21% Oxygen Compared with 100% Oxygen for Delivery of Isoflurane to Mice (*Mus musculus*) and Rats (*Rattus norvegicus*), pp. 148-154**

Domain 2: Management of Pain and Distress; T3

Primary Species: Mouse (*Mus musculus*) and Rat (*Rattus norvegicus*)

SUMMARY: At research institutions, isoflurane delivered by vaporizer to a face mask is the standard for rodent anesthesia. Pure oxygen is often used as the carrier gas, despite documented complications from longterm 100% oxygen use in humans and known occupational safety risks.

The authors examined the effect of anesthetic delivery gas on physiologic variables in mice and rats. Rodents were anesthetized for 60 min with isoflurane delivered in either 21% or 100% oxygen by means of a nose cone.

Studies in human medicine have shown that induction of anesthesia with oxygen concentrations below 60% is beneficial. CT imaging of human patients during inhalant anesthesia has shown areas of atelectatic lung that are dependent upon oxygen concentration.

Authors hypothesized that delivery of isoflurane in 21% oxygen would reduce atelectasis without significantly altering physiologic parameters in mice and rats.

Mice (n = 10) were anesthetized for 60 min through a nose cone by using a nonrebreathing system with isoflurane delivered in either 100% oxygen or 21% oxygen. Induction was made with 5% isoflurane delivered in either 100% oxygen or 21% oxygen, and they were maintained at 1.5-2%.

A femoral catheter was placed surgically to record blood pressure and facilitate blood collection. Respiratory rate and body temperature were taken immediately at onset of anesthesia. After 60 min of isoflurane anesthesia, body temperature, respiratory rate, and mean arterial blood pressure were measured. At the same time, an arterial blood sample was obtained to measure the pH, PaCO2, and PaO2. The animals were then allowed to recover and the recovery time calculated.

In 2 additional experiments, mice were similarly anesthetized for 60 min with isoflurane in 21% or 100% oxygen before being used to quantify atelectasis according to either pressure–volume hysteresis (n = 10) or histology (n = 5).

Same study design was used with rats, but physiologic parameters and blood gas values were measured at 15, 30, 45, and 60 min after the induction of anesthesia (n = 10). Time to anesthetic recovery was then determined, after which the rats were immediately euthanized. For rats, atelectasis was quantified by using μCT imaging.

In mice, the delivery gas did not affect physiologic parameters (body temperature, respiratory rate; mean arterial pressure; recovery time). Analysis of arterial blood showed that PaO2 was lower than expected for animals breathing 100% oxygen, demonstrating that they experienced relative hypoxemia. The animals given isoflurane in 21% oxygen had a normal A-a gradient (relative to the FiO2) of 19 mm Hg, but those that received 100% oxygen had an elevated A-a gradient (433 mm Hg). Therefore, the authors hypothesized that the cause of the relative hypoxemia in our 100% oxygen animals was a ventilation–perfusion mismatch due to atelectasis.

Histologic evaluation revealed greater atelectasis but no evidence of fibrosis in the 100% oxygen group compared with the 21% animals. Therefore, the most likely cause of the reduced lung compliance was atelectasis.

The likely mechanism of the increased atelectasis in animals that received isoflurane in 100% oxygen is absorption atelectasis.

Similar to findings from mice, no differences were seen in the body temperature or recovery time in rats between the 100% and 21% oxygen groups. Although statistical significance was not reached, the respiratory rates in rats given 100% oxygen showed a trend toward being lower than that in the 21% oxygen group at all time points

This finding is important because respiratory depression can lead to hypercapnia during anesthesia. Indeed, PaCO2 at 30, 45 and 60 min of anesthesia was higher in rats given 100% oxygen than in those that received 21% oxygen.

Unlike mice, rats exposed to 100% oxygen developed respiratory acidosis by 30 min of isoflurane anesthesia.

In addition, rats showed much greater variation in mean arterial blood pressure than did mice. Delivery of isoflurane in 100% oxygen was associated with mean arterial pressures that trended toward being higher than those in the 21% oxygen group (at 15, 30, and 45 min) and that were consistent with hypertension at all time points. However, mean arterial pressure at the 60-min time point was increased in the 100% oxygen group. Although the mechanism is not well understood, hypercapnia can stimulate the sympathetic nervous system, leading to peripheral vasoconstriction and subsequent hypertension.

At all-time points, rats given 21% oxygen had PaCO2 values that were consistent with previous reports from isoflurane anesthetized rats, whereas PaCO2 values were higher than expected in the 100% oxygen group.

Animals receiving isoflurane in 21% and 100% oxygen had a relative hypoxemia at every time-point. The PaO2 for both oxygen delivery concentrations was 3 times the FIO2.

Unlike mice, both groups of rats had A-a gradients that were higher than expected, suggesting that ventilation–perfusion mismatch is present regardless of the delivery gas used.

Morphometric quantitation of μCT images to compare total lung volume occupied by open airspace did not differ between the groups.

QUESTIONS

1.  T/F. When 100% oxygen is used to deliver isoflurane to a patient, the oxygen quickly diffuses across the alveoli into the blood stream, accelerating the rate of alveolar collapse. However, in room air (21% oxygen), oxygen shares alveolar space with less-soluble gases such as nitrogen, thus helping to retain alveolar patency

2.  T/F. benefits of decreased oxygen concentrations for isoflurane delivery are proven in all species

3. Abnormally increased A–a gradient suggests

1. Defect in diffusion, like atelectasis
2. Ventilation/perfusion ratio mismatch
3. Right-to-left shunt.
4. All of the above

4.  The normal ratio of PaO2/FiO2 is between

1. 1-2
2. 2-3
3. 4-5
4. 0.2-0.5

ANSWERS

1. T

2. F

3. d

4. a

**Martin-Flores et al. Effects of Buprenorphine, Methylnaltrexone, and Their Combination on Gastrointestinal Transit in Healthy New Zealand White Rabbits, pp. 155-159**

Domain 3: Research

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: In this study, 8 male New Zealand White rabbits were placed in a crossover randomized study looking at the effects of buprenorphine, methylnaltrexone and their combination on gastrointestinal transit time. Buprenorphine is used commonly in rabbits, but is concerning because of the associated gastrointestinal stasis. There were 4 treatment groups: saline, buprenorphine, methylnaltrexone, and buprenorphine with methylnaltrexone. Rabbits were given barium filled spheres via an orogastric tube, then received 4 treatments, each 12 hours apart. For the next 5 days rabbits were weighed, as well as food and water intake measured, and feces weighed and radiographed. Only 1 animal showed abnormal signs during the study, a rabbit in the burprenorphine group was dehydrated on day 6.

On data analysis, the barium spheres appeared at 18 hours for the control and methylnaltrexone groups, 30 hours for the buprenorphine group, and 24 hours for the combination group. However, not all spheres were recovered, and in some animals no spheres were recovered. Fecal weight was lowest for all groups on day 1; between groups methylnaltrexone had the highest fecal weight and buprenorphine and the combination group had the lowest fecal weights. There was no change in body weight for any treatment group. Water and food intake decreased buprenorphine and the combination group, and increased for the methylnaltrexone group.

Overall, buprenorphine increased gastrointestinal transit time, decreased fecal output, and decreased food and water intake; the addition of methylnaltrexone was not sufficient to counteract the negative gastrointestinal effects of buprenorphine. Effects of buprenorphine on fecal production lasted to day 5, even though it was only dosed out to day 2. Methylnaltrexone alone increased food and water intake and fecal weight.

QUESTIONS

1. What is the concept behind using the combination of buprenorphine and methylnaltrexone?

2. What are the expected side effects with buprenorphine?

3. What are the classes of opioids based on receptors?

ANSWERS

1. Because methylnaltrexone does not cross the blood brain, it should not affect the sedation and analgesic effects of buprenorphine; however, it will still act as an antagonist peripherally, specifically on gastrointestinal stasis.

2. Respiratory depression, sedation. In dogs: salivation, bradycardia, hypothermia, agitation, dehydration, miosis. In cats: mydriasis, behavioral changes. In horses: excitement, diminished gut sounds. (Plumb’s)

3. Receptors: (lists from Anesthesia and Analgesia in Laboratory Animals, 2008)

a.  Mu: most clinically relevant; supraspinal

i.  Agonists: morphine, methadone, etorphine, levorphanol, fentanyl, sufentanil

ii.  Partial agonists: butorphanol, buprenorphine, pentazocine

iii.  Antagonists: naloxone, naltrexone, diprenorphine, nalorphine, nalbuphine

b. Kappa: spinal

i.  Agonists: from (strong to weak) butorphanol and etorphine, pentazocine and nalbuphine, morphine and sufentanil

ii. Antagonists naltrexone and diprenorphine, buprenorphine and naloxone

c. Delta: poor analgesic, may modify Mu

i.   Agonists: etorphine, sufentanil

ii.  Antagonists: diprenorphine, naloxone, naltrexone

d.  NOP: anti-opioid effects

**Cary et al. Pharmacokinetic Profiles of Meloxicam and Sustained-released Buprenorphine in Prairie Dogs (*Cynomus ludovicianus*), pp. 160-165**

Domain 2; T2

Tertiary Species: Other Rodents - Prairie Dog (*Cynomys ludovicianus*)

SUMMARY: This study evaluated the pharmacokinetic profiles of two doses of meloxicam (0.2 mg/kg SC and 4 mg/kg SC) and two doses of buprenorphine SR (0.9 mg/kg SC and 1.2 mg/kg SC). Results showed that the low dose of meloxicam did not maintain proposed therapeutic levels as reported in other species for 24 hours despite being the recommended dose in prairie dogs. The high dose of meloxicam did maintain the maximal proposed therapeutic levels (0.9 μg/mL) past the 72-hour time point. The buprenorphine SR data showed that both doses, which were chosen based on previous studies in other rodents maintained plasma levels above the suggested therapeutic level (0.001 μg/mL) beyond the 96-hour time point. As a result, the revised proposed dosage of buprenorphine SR is 0.9mg/kg every 96 hours for prairie dogs. The injection site reactions seen in a subset of the prairie dogs in this study highlight concerns regarding the use of buprenorphine SR. To reduce the incidence of lesion development it is suggested to slowly withdraw the needle after injection and to pinch the injection site for 15 seconds.

QUESTIONS

1. Prairie dogs are used to study what disease model?

2. What are three techniques that can be used to achieve multi-modal analgesia?

ANSWERS

1. Monkeypox

2. a. Combine drugs with different pharmacologic mechanisms. b. Use different routes of administration for drugs with similar modes of action. c. Use drugs that may counteract the potential side effects of each drug.

***Experimental Use***

**Manuel et al. Procedure for Horizontal Transfer of Patient-Derived Xenograft Tumors to Eliminate *Corynebacterium bovis*, pp. 166-172**

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

Primary species: Mouse (*Mus musculus)*

SUMMARY: The article described how 61 unique human patient-derived xenograft (PDX) tumors were successfully harvested from immunodeficient mice infected with *Corynebacterium bovis,* and transplanted into *C. bovis* free immunodeficient mouse colonies, without transfer of *C. bovis*.  The protocol used minor enhancements to traditional aseptic surgical techniques in combination with bioexclusion principles.  The procedure was implemented in the fall of 2012 and no recipient mice have tested positive for *C. bovis* since then.

The successful technique used two people, one to harvest tissue and one to receive tissue and implant into *C. bovis* free mice.  Briefly, a freshly euthanized mouse was dipped in 2% chlorhexidine gluconate for at least a 5-minute contact time with skin.  Two pair of sterile instruments were used in an aseptic biosafety cabinet to harvest tissue.  One set of instruments touched external mouse surfaces and one set touched only subcutaneous tissue.  Tissue was placed into transfer media, the vial thoroughly disinfected, and taken to a clean environment.  Tissues were processed for implantation and the second person used sterile technique to implant tissues.

QUESTIONS

1.  T/F. A dry sterile swab may be used on the body surface (skin) and oral cavity to detect *C. bovis* using qPCR analysis.

2.  Sterile tip instrument technique requires use of which of the following to complete the procedure properly?

a.  Sterile gloves

b.  Sterile drape

c.   Aseptic surgery site preparation

d.   Bead sterilizer or autoclave

e.   All of the above

3.   Clinical symptoms of *C. bovis* infection in athymic nude mice may include which one of the following?

a.  Scaly skin

b.   Decreased body condition

c.  Lethargy and dehydration

d.   All of the above

4.  T/F. *C. bovis* is a common contaminant of susceptible mouse populations and tumors harvested from the mice, even if tissue is frozen.

ANSWERS

1.   T

2.  c & d

3.  d

4.   T

**Gordon et al. A Device that Allows Rodents to Behaviorally Thermoregulate when Housed in Vivariums, pp. 173-176**

Domain 4: Animal care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: A variety of situations potentially warrant keeping mice at a warmer, thermoneutral environment, including during the post-operative period, during administration of a compound that might elicit a hypothermic response, etc. The authors created a false floor for a standard IVC cage, allowing for an aluminum panel insert to be placed with a hand warmer underneath to create a level area of warmth within the cage by conductive heating. The aluminum insert maintained a heat of >30\*C within the cage for 13h in a vivarium maintained between 20-22\*C. Mice preferred to sleep over the warm aluminum plate during the light hours, and moved off the plate during the dark (awake) hours. Each complete unit is estimated to cost $10.

QUESTION

1. What is thermoneutral zone in a mouse in the light phase?

ANSWER

1. 30-32°C

**Lee et al. Morphologic and Doppler Findings from Hepatic Ultrasonography of Normal Cynomolgus Monkeys (*Macaca fascicularis*), pp. 177-180**

Domain 3: Research; K3 animal models

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Doppler ultrasonography plays a key role in postoperative monitoring of liver transplant recipients, allowing for patency evaluation of the portal vein, hepatic artery, and hepatic vein.  The current study evaluated the anatomic and normal sonographic features of the liver in normal cynomolgus monkeys (*Macaca fascicularis*) using 2D and Doppler ultrasonography to be used as references for future studies.   Measurements were obtained from fasted, sedated animals in a supine position with a 9L linear transducer and the LOGIQ E9 system by the same operator.  Animals were sedated with Ketamine (7mg/kg) rather than general anesthesia with isoflurane to avoid altered systemic hemodynamics.  Vessel diameter was obtained with by using the electric caliper incorporated in the ultrasonic device with the transducer placed transversely to the hepatic pedicle just below the caudal rib.  Parenchymal echogenicity of the liver was compared with that of the right kidney with the transducer parallel to the hepatic pedicle.  Flow rates of vessels were obtained with the transducer parallel to the hepatic pedicle with Doppler waveforms obtained by insonating the vessels at a 60° angle to the ultrasound beam.

Normal liver parenchyma was more hypoechoic than kidney parenchyma and showed homogenous echogenicity.  Refer to Table 1 for vessel and common bile duct diameter measurements.  The right posterior portal vein and right anterior portal vein branched from the main portal vein (MPV) in both bifurcation and trifurcation patterns.  Doppler ultrasonography showed a pulsatile waveform of the hepatic artery, triphasic waveform of the hepatic vein, and monophasic waveform of the portal vein in most cases.  Some cases exhibited phasicity of the portal vein waveform due to respiration or pulsation of the adjacent hepatic artery.  Mean peak systolic velocity of the proper hepatic artery (PHA) was 75.4 cm/s with a resistance index of 0.54, and mean velocity of the MPV was 19.3cm/s.  Diameters of the MPV and suprahepatic inferior vena cava were larger in macaques weighing > 4.5kg; however, diameters of the common bile duct and other vessels did not differ significantly by body weight.

QUESTIONS

1. A large antegrade systolic and diastolic waveform with a retrograde wave due to backward transmission from right atrial pressure changes during the cardiac circle represents what type of waveform on Doppler ultrasonography?

a.   Pulsatile waveform

b.  Biphasic waveform

c.  Triphasic waveform

2. Which two vessels were found to have a larger diameter for animals > 4.5kg?

a.   Main portal vein and suprahepatic vein

b.  Main portal vein and proper hepatic artery

c.  Suprahepatic inferior vena cava and suprahepatic vein

d. Suprahepatic inferior vena cava and infrahepatic inferior vena cava

ANSWERS

1. c. Triphasic waveform

2. a. Main portal vein and suprahepatic vein

**Rapp-Santos et al. Comparison of Saliva Collection Methods for the Determination of Salivary Cortisol Levels in Rhesus Macaques (*Macaca mulatta*), Cynomolgus Macaques (*Macaca fascicularis*), and African Green Monkeys (*Chlorocebus aethiops*), pp. 181-189**

Primary Species: Macaques (*Macaca spp.*)

Tertiary Species: Other Nonhuman Primates (African Green Monkeys – *Chlorocebus aethiops*)

SUMMARY

Glucocorticoid measurement remains one of the most frequently evaluated biomarkers of stress in mammals, including NHP. Investigators have collected a variety of bio-samples to measure cortisol. Serum or plasma cortisol is one of the most widely used biomarkers for stress in mammals including NHP. Saliva collection represents and alternative non-invasive method to measure cortisol.

The objectives of the current study were to;

1.      Compare salivary cortisol levels, collection volume, and ease of use for 2 different methods of saliva collection – passive drool (PD) and a commercially available salivary collection device (SS) in 3 NHP species.

2.      Demonstrate the correlation between serum and salivary cortisol levels to determine whether salivary cortisol measurements accurately reflect serum cortisol.

3.      Determine the range of cortisol levels in both the serum and saliva of anaesthetized healthy adult cynomolgus macaques, rhesus macaques and African green monkeys.

The salivary collection device (SS) was easy to use and was more reliable in collecting sufficient volume of saliva compared with passive drool (PD), and the resulting salivary cortisol values demonstrated stronger correlation with serum cortisol concentration in all species. The determination of salivary cortisol might serve as a useful indicator of stress for NHP in a variety of situations. Further studies are needed to evaluate salivary cortisol from voluntary saliva samples collected with the SS.

QUESTIONS

1.  True or False. Diurnal variability has been demonstrated in male rhesus monkeys.

2.  True or False. Variation has also been observed in rhesus macaques according to male dominance, with lower-ranked animals having higher mean cortisol levels.

3.  Which advantages does saliva collection for cortisol measurement have over other bio-samples?

a. Cortisol is highly lipid-soluble and rapidly diffuses from the blood into the acinar cells of the salivary glands at a constant 10% to 15% fraction of circulating levels.

b. In contrast to serum or plasma, which allows only total cortisol measurement, salivary cortisol analysis represents the unbound biologically active cortisol.

c. Free salivary cortisol appears to be independent of saliva flow rate.

d. Peak salivary cortisol levels lag behind serum levels after the onset of the stressor, reducing the potential confounders of researcher presence and physical restraint and allowing better assessment of baseline cortisol.

e. All of the above

ANSWERS

1.  True

2. True

3.  e

**CASE REPORTS**

**Wasson. Retrospective Analysis of Reproductive Performance of Pair-bred Compared with Trio-bred Mice, pp. 190-193**

Domain 4: Animal care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: A retrospective analysis of mouse breeding data was performed to determine the effects of 2 breeding schemes, i.e. pair-bred and trio-bred mice, on reproductive performance. The number of pups weaned per female, number of litters produced per female, and number of pups weaned per breeding scheme were analyzed.

Fifty-three inbred genetically altered mouse strains on C57Bl/6, BALB/c and C3H backgrounds were sourced from a variety of sources or bred in-house. Breeding pairs or trios were setup at 6 – 8 weeks of age and remained together for approximately 144 days. Breeding data from 275 breeding units were retrieved from a LAM software program. The data covered a period of 21 months. Cage densities were higher than those recommended in the Guide for the Care and Use of Laboratory Animals. Pups deemed too small to wean at 21 days were allowed to stay with parents for additional days as needed. Pairs or trios with no pups 45d after setup were excluded and replaced. Breeding units that had been established for less than 21d when data were collected were also excluded.

The room temperature was 20 – 26°C, humidity 30-70%, and a 12:12-h light: dark cycle. Mice were housed in IVCs and provided with an irradiated corncob bedding, nesting material, RO purified water and rodent chow.

Typically, cages were changed every 2 weeks. Cages with new litters were left undisturbed for 3 – 5 days.

The Satterthwaite approximation t-test for independent samples with unequal variance was used for analyses. There was no statistical difference in the number of pups weaned per female or the number of litters produced per female. Approximately one pup more was weaned for trio-bred litters compared with pair-bred litters.

Floor space is not a critical factor for optimal reproductive performance.

QUESTION (True or False)

1. The reproductive performance of trio-bred mice is similar to pair-bred mice.

ANSWER  
1. True

**Leduc et al. What Goes Around Can Come Around: An Unexpected Deleterious Effect of Using Mouse Running Wheels for Environmental Enrichment, pp. 194-201**

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Providing running wheels has become a more common method of providing environmental enrichment to laboratory animals. Wheel running can be beneficial to many physiologic systems and can have a beneficial effect on metabolism and aging. It can also be used to simulate the effects of exercise in models of various human conditions. Wheel running has been associated with positive effects on mouse and rat models, however, it has also been shown to lead to body changes, increased aggression and addiction-like behavior.  Wheel running is commonly reported to attenuate stereotypic behavior in laboratory mice, although not in deer mice.  The presence of a wheel has been associated with increased aggression in male mice.  Female mice have also been shown to compete over environmental enrichment, including a running wheel.

Circling behavior may be a form of stereotypic behavior, but can also be attributed to defects in vestibular function of the inner ear which interfere with the ability to sense gravity.  Some circling rodent models also show defects in the dopamine signaling pathway which suggests that circling behaviors due to vestibular dysfunction may act in part through changes in the dopamine system.

This case study showed that while voluntary wheel running can be associated with benefits in rodent models of human disorders, there can also be negative effects in certain strains of mice.

FVB;129/Hemc male mice were weaned into social groups of 2 to 5 animals per cage and were provided with igloo style running wheels.  A percentage of male mice housed with running wheels began to develop circling behavior, which was obvious and permanent.  In addition, males in cages with wheels also showed a predisposition to fighting. Necropsy and further testing showed that the circling mice have significantly lower levels of dopamine and its metabolite DOPAC in whole-brain extracts.

QUESTIONS (True or False)

1. Running wheels always have a positive effect on mouse behavior.

2.  Circling behavior in some strains of mice can be associated with decreased levels of dopamine.

ANSWERS

1.   False

2.   True

**Manuel et al. Detection and Elimination of *Corynebacterium bovis* from Barrier Rooms by Using an Environmental Sampling Surveillance Program, pp. 202-209**

SUMMARY: Rodent health-monitoring programs based on sampling an IVC system’s exhaust air dust (EAD) has enhanced and even replaced traditional sentinels for some rodent pathogens. EAD testing by qPCR assay is an optimal surveillance method for the rapid detection of *Corynebacterium bovis*-infected immunodeficient mice. Here authors demonstrate that an active EAD surveillance program for *C. bovis* can be used to maintain nude mice *C. bovis*-free after the transition from historically enzootically infected colonies. During 3 events over 3 y, rapid detection of infection, elimination of infected mice, aggressive quarantine measure, and local decontamination prevented the spread of *C. bovis* within 2 barrier rooms. In total, 4 cages of infected nude mice were identified and removed, preventing the spread of infection to 469 other cages of immunodeficient mice. In addition, authors present data regarding a refinement to EAD testing which enables row-specific surveillance of and IVC rack. This technique systemically decreases the amount of testing required to locate an individually infected cage. Due to their ability to rapidly detect and localize an infected cage, the authors were able to investigate the route of *C. bovis* introduction into their barrier rooms. Epidemiologic investigation suggested that the transmission of *C. bovis*occurred through contained, cryopreserved, patient-derived xenograft tumor tissue. This previously unknown source of *C. bovis* can infect mice used to propagate these tumors. Together, these data demonstrate that a remediation program that combines rapid detection, test-and-cull, and local decontamination under quarantine conditions can eliminate *C. bovis*from a mouse colony.

QUESTIONS

1. Briefly describe methods to both prevent/treat *C. bovis* in a rodent facility.

2. What clinical signs are associated in mice with *C. bovis* infection in mice?

ANSWERS

1. Health monitoring for *C. bovis*should be performed regularly for nude, hairless, and SCID animals. Animals should be sourced from regularly tested colonies. Aseptic hysterectomy rederivation with fostering onto clean females or embryo transfer rederivation will remove *C. bovis*from a colony. Animals may also be treated with antibiotics to reduce clinical signs while waiting to rederive or receive new animals. *C. bovis*isolates have been shown to be sensitive to tetracycline, enrofloxacin, and ampicillin.

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.

**Head et al. Techniques for Nonterminal Blood Sampling in Black-Tailed Prairie Dogs (*Cynomys ludovicianus*), pp. 210-213**

Domain 3: Research

Tertiary Species: Other Rodents – Black-Tailed Prairie Dog (*Cynomys ludovicianus*)

SUMMARY

Introduction: No studies are available currently that report the total blood volume of prairie dogs but for a similar species it was 7-8% of body weight. The recommended maximal proportions of the total blood volume that can be collected with a minimal likelihood of adverse side effects are 0.5% daily, 5% weekly, 7.5% biweekly, and 10% monthly. Blood samples of 1 mL or larger can be obtained from the jugular vein, femoral vein, or the cranial vena cava of black-tailed prairie dogs. Peripheral sites such as the cephalic vein, saphenous vein, and tarsal vein, are useful for smaller blood samples. General anesthesia is commonly required and blood samples should be collected immediately after anesthetic induction. Peripheral veins can be preheated and locally occluded with a tourniquet to enhance their visualization.

Sites for Blood Collection

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SITE | AMOUNT | NEEDLE GAUGE | SYRINGE | POSITION | NOTES |
| Jugular vein | 6 mL | 23-25 | 1-6 mL | Dorsal recumbency. Forelimbs extended caudally or positioned upright over the edge of a table, with forelimbs pointed toward floor | Use finger pressure to occlude jugular vein at the level of the shoulder. |
| Femoral (medial saphenous) vein | 1-3 mL | 25-27 | 1-3 mL | Lateral or dorsal recumbency. | Requires tourniquet or pressure on inguinal region. Femoral vein is located centrally on the medial aspect of the thigh |
| Cranial vena cava | 6 mL | 25-27 | 1-6 mL | Dorsal recumbency. Head extended cranially and forelimbs extended distally. | Palpate the manubrium and clavicle and introduce needle just lateral to the manubrium and into the clavicular notch at 45degree angle. |
| Cephalic vein | 1 mL | 25-27 | 1 mL |  | Should be preheated and occluded with tourniquet |
| Lateral saphenous vein | 1 mL | 25-27 | 1 mL | Lateral recumbency | Should be preheated and occluded with tourniquet |
| Tarsal vein | 100 uL | 27 | Insulin syringe or capillary tube | Lateral recumbency | Should be preheated and occluded with tourniquet |

QUESTIONS

1.   True or False: Of the 5 prairie dog species, black-tailed prairie dogs are the most common, both in the wild and in captivity.

2. Prairie dogs are a useful animal model for human:

a.  Type 1 diabetes

b. Gallbladder disease

c. Storage diseases

d. Pancreatic neoplasia

e.  Blindness

ANSWERS

1. True

2.  b