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**ORIGINAL RESEARCH**

***Biology***

**Otto et al*.* Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (*Mus musculus*), pp. 375-386**

Domain 3:  Research

Primary Species: Macaques (Macaca spp.)

SUMMARY: Clinical chemistry of blood serum or plasma is a common and key research tool.  As genetic variations between mouse strains can impact the normal range of clinical chemistry parameter results.  The current study evaluated common clinical chemistry responses in three separate strains of mice, the closely related C57BL/6J and the C56BL/6N, and the more distantly related C3HeB/FeJ.  Mice of both sexes and ranging in age from 90 to 135 days were tested.  Sex related differences were found with female mice displaying significantly higher values for chloride, AST, ALP and albumin while male mice displayed higher values for sodium, potassium.  Correlation of results with age found statistically significant differences.  However, the differences were found to have a low coefficient of determination such that the biological relevance of age related differences is unclear.  Strain differences were minor between C57BL/6J and C57BL/6N, and more pronounced when compared to C3HeB/FeJ, particularly for albumin, total protein, potassium, triglyceride and cholesterol.  In general differences between sexes were greater than those between strains or based upon age.

QUESTION

1. Based upon the current study what was the most important predictor of differences, age, sex, or strain?

ANSWER

1. Sex

***Reproduction***

**Whitaker et al. Effects of Enrichment and Litter Parity on Reproductive Performance and Behavior in BALB/c and 129/Sv Mice, pp. 387-399**

Primary Species: Macaques (Macaca spp.)

SUMMARY: The effects of adding species-appropriate environmental enrichment items to the breeding cages of BALB/cAnNCrl and 129S2/SvPasCrl mice were explored. The 3 enrichment conditions were: 1) cotton nesting material, 2) nesting material with paper shelter and rolled paper bedding, and 3) igloo dome with an exercise wheel in addition to the other nesting materials. Litter size, litter survival to weaning age, average pup weight at weaning, and inter-litter interval were used to evaluate reproductive performance. The mice behavior was explored using elevated plus maze test, open field, social approach, marble burying, and acoustic startle. Results found that enrichment significantly affected anxiety-like behavior and sociability which was dependent on strain and sex. Liter parity had greater effects on reproductive parameters than the enrichment conditions. The 129 mice weaned less pups in the nest only enrichment and BALB/c mice had longer inter-litter interval under the nest-only condition. This indicated that both the nest+shelter and nest+shelter+wheel conditions were better than the nest-only condition in both strains. Male mice exposed to the nest+shelter+wheel items had behavior profiles more comparable to that of wild-type strain B6 which indicates less anxiety. 129 male mice from the nest+shelter+wheel groups showed greater control over risk-taking behaviors. Overall results found that enrichment can both increase and decrease anxiety-like behaviors and sociability. The effects of the natal cage environment on reproduction did not persist from offspring to the adult cage. This means that whatever enrichment that is current in the cage overshadows any former effect from the natal cage. This also means that transporting young mice from one facility to another does not affect the adult reproductive success.

QUESTIONS

1. T/F. Enrichment had a greater effect on reproductive parameters compared to litter parity.

2. Which enrichment condition was found to have more positive behavioral effects?

a. Nesting only

b. Nesting + shelter

c. Nesting + shelter + wheel

ANSWERS

1. F. Litter parity has a greater effect on reproductive parameters.

2. b and c

**Esmail et al. Generating Chimeric mice by Using Embryos from Nonsuperovulated BALB/c Mice Compared with Superovulated BALB/c and Albino C57BL/6 Mice, pp. 400-405**

Domain 3: Research

Primary Species: Mouse (Mus musculus)

SUMMARY:  The inbred C57BL/6 strain has become very popular for the generation of new animal models, and numerous robust, germline-competent embryonic stem (ES) cell lines sequencing of the C57BL/6 mouse genome are now available.  Microinjection of mutant ES cells into host blastocysts produces chimeric mice, and because the contribution of the ES cells to the offspring can be assessed visually according to coat color differences between ES cells and the embryo donor, ES cells derived from C57BL/6 black mice are often injected in host embryos produced by albino inbred mice. Tyrosinase-mutant albino C57BL/6 and naturally tyrosinase deficient albino BALB/c mice have both been used to produce host embryos for injection with ES cells during the generation of chimeric mice. Both strains are often superovulated to synchronize estrus cycles and produce increased numbers of blastocysts when bred, but substrain- and age-associated differences lead to variable effectiveness in generating embryos. The reliable generation of high-percentage chimeras from gene-targeted C57BL/6 embryonic stem cells has proven challenging, despite optimization of cell culture and microinjection techniques. Seeking to improve the efficiency of this procedure, the authors of this study compared the generation of chimeras by using 3 different inbred, albino host, embryo-generating protocols:

•        4-wk-old BALB/cAnNTac (BALB/c) donor mice with superovulation

•        12-wk-old BALB/c donor mice without superovulation

•        4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice with superovulation

Key parameters measured included the average number of injectable embryos per donor, the percentage of live pups born from the total number of embryos transferred to recipients, and the number of chimeric pups with high embryonic-stem cell contribution by coat color.  Albino B6 donors produced significantly more injectable embryos than did BALB/c donors, but 12-wk-old BALB/c donors produced high-percentage (at least 70%) chimeras more than 2.5 times as often as did albino B6 mice and 20 times more efficiently than did 4-wk-old BALB/c donors. These findings clearly suggest that 12-wk-old BALB/c mice be used as blastocyst donors to (1) reduce the number of mice used to generate each chimera, (2) reduce the production of low-percentage chimeras, and (3) maximize the generation of high-percentage chimeras from C57BL/6 embryonic stem cells.  The adoption of these donor blastocyst protocols depend on the transgenic facility and experimental needs.  To use mature BALB/c female mice for donor blastocysts, a facility must maintain a population of mice that is much larger than the minimum, given that natural estrus cycling must be accommodated, and the necessary size of the colony may mean that some animals are not used. If a facility cannot maintain a large BALB/c colony, albino B6 mice may be a viable option for the generation of donor blastocysts, given that this strain yielded higher fertility and increased numbers of blastocysts relative to the BALB/c experimental groups.

QUESTIONS

1. According to this study, which of the following blastocyst donors produced significantly more injectable embryos?

a. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITH superovulation

b. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITHOUT superovulation

c. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITH superovulation

d. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITHOUT superovulation

e. 12-wk-old BALB/c donor mice WITH superovulation

f. 12-wk-old BALB/c donor mice WITHOUT superovulation

2. According to this study, which of the following blastocyst donors best reduced the number of mice used to generate each chimera?

a. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITH superovulation

b. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITHOUT superovulation

c. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITH superovulation

d. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITHOUT superovulation

e. 12-wk-old BALB/c donor mice WITH superovulation

f. 12-wk-old BALB/c donor mice WITHOUT superovulation

3. According to this study, which of the following blastocyst donors best reduced the production of low-percentage chimeras?

a. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITH superovulation

b. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITHOUT superovulation

c. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITH superovulation

d. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITHOUT superovulation

e. 12-wk-old BALB/c donor mice WITH superovulation

f. 12-wk-old BALB/c donor mice WITHOUT superovulation

4. According to this study, which of the following blastocyst donors best maximized the generation of high-percentage chimeras from C57BL/6 embryonic stem cells?

a. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITH superovulation

b. 4-wk-old BALB/cAnNTac (BALB/c) donor mice WITHOUT superovulation

c. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITH superovulation

d. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITHOUT superovulation

e. 12-wk-old BALB/c donor mice WITH superovulation

f. 12-wk-old BALB/c donor mice WITHOUT superovulation

5. According to this study, what type of female mice should be used as blastocyst donors in facilities that are able to maintain a large enough population of mice to allow natural estrus cycling?

a. Immature BALB/c donor mice WITH superovulation

b. Immature BALB/c donor mice WITHOUT superovulation

c. Immature albino B6 donor mice WITH superovulation

d. Immature albino B6 donor mice WITHOUT superovulation

e. Mature BALB/c donor mice WITH superovulation

f. Mature BALB/c donor mice WITHOUT superovulation

6. According to this study, what type of female mice should be used as blastocyst donors in facilities that are NOT able to maintain a large enough population of mice to allow natural estrus cycling?

a. Immature BALB/c donor mice WITH superovulation

b. Immature BALB/c donor mice WITHOUT superovulation

c. Immature albino B6 donor mice WITH superovulation

d. Immature albino B6 donor mice WITHOUT superovulation

e. Mature BALB/c donor mice WITH superovulation

f. Mature BALB/c donor mice WITHOUT superovulation

ANSWERS

1. c. 4-wk-old C57BL/6NTac-Tyrtm1Arte (albino B6) donor mice WITH superovulation

2. f. 12-wk-old BALB/c donor mice WITHOUT superovulation

3. f. 12-wk-old BALB/c donor mice WITHOUT superovulation

4. f. 12-wk-old BALB/c donor mice WITHOUT superovulation

5. f. Mature BALB/c donor mice WITHOUT superovulation

6. c. Immature albino B6 donor mice WITH superovulation

***Husbandry***

**Boivin et al. Responses of Male C57BL/6N Mice to Observing the Euthanasia of Other Mice, pp. 406-411**

Domain 2

Primary Species: Macaques (Macaca spp.)

SUMMARY: The AVMA Panel on Euthanasia recommends that sensitive animals should not be present during the euthanasia of others, especially of their own species, but does not provide guidelines on how to identify a sensitive species. To determine if mice are a sensitive species the authors reviewed literature on empathy in mice, and measured the cardiovascular and activity response of mice observing euthanasia of conspecifics. 16 wk old C57BL/6N were fitted with telemetry pressure transmitting probes to monitor their response to observing CO2 euthanasia and decapitation. Mice observing CO2 euthanasia had no increase in cardiovascular parameters or activity, while mice observing decapitation had an increase in all values. Researchers noted that a similar increase in response was seen during mock decapitations where no animals were handled or euthanized. They presumed that the increase can be attributed to the noise of the guillotine. Several studies provide evidence that rodents respond to alterations in their environment through pheromones, odors, vocalization, and sight.  This paper supports the conceptual idea that mice are both a sensitive species and display empathy, but showed no signaling of stress during observation of euthanasia procedures in this study.

QUESTIONS

1. Cervical dislocation is acceptable with conditions for mice and rats:

a. >200 g

b. <200 g

c. >2kg

d. >20kg

2. What is the preferred physical method of euthanasia when immediate fixation of brain metabolites is required for research purposes?

a. Decapitation

b. Cervical dislocation

c. Pentobarbital

d. Focused beam microwave irradiation

ANSWERS

1. b

2. d

**Martin et al. Cost and Effectiveness of Commercially Available Nesting Substrates for Deer Mice (*Peromyscus maniculatus*), pp. 412-218**

Domain 4: Animal Care

Tertiary Species: Other Rodents

SUMMARY: Deer mice are a very common rodent species in North America and their use in biomedical research is increasing. Housing for deer mice has historically mimicked housing for *Mus* species, however, there is little data to prove these methods are appropriate. Commercially available nesting substrates were provided several types of mouse caging configurations- all male, all female, and breeding pairs. Nesting substrates were evaluated 24h after cage change and scored for complexity. Dispersed paper had the lowest score and was the cheapest. The other types did not differ in nest complexity. Due to the reasonable cost and quality of nest, brown crinkle paper was the most practical substrate for use as enrichment for deer mice in the laboratory setting.

QUESTIONS

1. Deer mice (*Peromyscus* spp) are natural hosts for which infectious disease?

a. Marburg virus

b. Hanta virus

c. SARS

d. *Coxiella burnetii*

2. True or False: Laboratory bred deer mice are not covered by the AWA and its Regulations.

3. True or False: Deer mice are reported to be poor mothers, but complex nest building is associated with good mothering ability.

ANSWERS

1. b

2. False

3. True

***Management***

**Steelman and Alexander. Laboratory Animal Workers’ Attitudes and Perceptions Concerning Occupational Risk and Injury, pp. 419-425**

Domain 5, Task 3

SUMMARY: Little is known regarding the risk perceptions and attitudes of laboratory animal care workers toward biologic safety. The purpose of this descriptive study was to assess the attitudes and perceptions of laboratory animal workers toward occupational and injury risk. Subscribers to the Comp Med and TechLink listservs (n = 4808) were surveyed electronically, and 5.3% responded; data from 215 respondents were included in the final analysis. Primary variables of interest included AALAS certifications status, level of education, and responses to Likert-scale questions related to attitudes and perceptions of occupational risk and injury. Nonparametric (χ2) testing and measures of central tendency and dispersion were used to analyze and describe the data. According to 88.6% of respondents, biologic safety training is provided with information about zoonotic diseases of laboratory animals. Level of education was significantly related to perception of importance regarding wearing personal protective equipment. Participants indicated that appropriate support from coworkers and management staff is received, especially when performance and perception are hindered due to stress and fatigue. Laboratory animal staff are susceptible to injury and exposure to dangerous organisms and toxic substances. For this reason, to maximize safety, yearly biologic safety training should be provided, the importance of protective equipment adherence strengthened, and the culture of safety made a priority within the institution.

Take-Home Points

* + - Prevention of injury from zoonotic exposure one of the objectives in a biological safety program
		- Occupational health and safety policies perform the following functions:

o   Enhance health

o   Facilitate pursuit of health

o   Provide economic advantages to individuals, communities, and organizations

* Occupational Safety and Health Administration and the Institutional Biosafety Committee require specialized training for employees working with hazardous agents
* Risk – the likelihood of an occurrence combined with the consequence; ultimately anything that can cause disease in other living organisms

o   Consequences include infection or allergies

o   Risks can be direct (e.g., infection) or indirect (e.g., damage to the environment)

* Toxic agents can enter the body through multiple means:

o   Skin contact, inhalation, ingestion, gas and vapor, particulate matter (dust, fume, mist, fog)

* Attitudes and perception of risks influence actions of staff members while at work

o   Attitudes – encompass long-standing evaluations of people and ideas

o   Perception – process of interpreting and organizing sensations to produce meaningful experiences

QUESTIONS

1.   Define ‘risk,’ as stated in this paper.

2.   Which two organizations require specialized training for employees who work with hazardous agents/substances?

3.   Define the Likert-type scale.

ANSWERS

1.  Risk - the likelihood of an occurrence combined with the consequence

2.  Occupational Health and Safety Administration, and the Institutional Biosafety Committee

3.  Psychological scales that use fixed choice response formats; they are designed to measure attitudes or opinion (agree/disagree/neutral)

***Anesthesia***

**Siriarchavatana et al. Anesthetic Activity of Alfaxalone Compared with Ketamine in Mice, pp. 426-430**

Domain 2: Management of Pain/Distress; T3: Anesthesia

SUMMARY

Alfaxalone:

* + Recently-approved (US) anesthetic for dogs and cats
	+ Neuroactive steroid agent
	+ Binds GABA receptor (subtype A)
	+ Early versions (1971) insoluble in water; oil versions had anaphylactoid reactions
	+ New version complexed with 2-hydroxypropyl-β-cyclodextrin, improving solubility
	+ Data published in cats/dogs, pigs, horses, and rats (IV methods)
	+ DEA Schedule V controlled substance

Evaluation of alfaxalone as single drug (mice)

* + Loss of righting reflex (LORR) observed at 40-80 mg/kg IP administration
	+ Toe pinch reflex and muscle tone remained present at 80 mg/kg
	+ Onset faster (~2 min) and duration of action longer (~55 min) compared to ketamine
	+ Hopping, scratching, jerking movements observed during induction

Evaluation of alfaxalone combined with xylazine (80 mg/kg alf. + 10 mg/kg xyl.)

* + Complete absence of reflexes within 2.5 minutes
	+ Duration of action ~80 minutes
	+ Induction behaviors reduced compared to alfaxalone alone
	+ Recovery safe and return to normal behavior in all animals studied

QUESTIONS

1. What is the mechanism of action of xylazine?  Ketamine?

2.  When discussing inhaled anesthetics, what does MAC stand for? How is it defined?

ANSWERS

1.  Xylazine is an alpha-2-adrenergic agonist.

 Ketamine is known primarily as an NMDA receptor antagonist, though the complete pharmacology is more complex (weak opioid receptor agonist, among others)

2.  MAC = Minimum Alveolar Concentration, the concentration of anesthetic drug in the lungs required to prevent nociception in 50% of the subject group.

**Malavasi et al. Cardiopulmonary Effects of Constant-Rate Infusion of Lidocaine for Anesthesia during Abdominal Surgery in Goats, pp. 431-435.**

Secondary Species: Goat (Capra hircus)

SUMMARY: Lidocaine is commonly used in ruminants but anecdotal history of being toxic to goats. To evaluate lidocaine’s effects on selected cardiopulmonary parameters, Isoflurane-anaesthetized adult goats (n = 24) undergoing abdominal surgery received a loading dose of lidocaine (2.5 mg/kg) over 20 min followed by constant-rate infusion of lidocaine (100 µg/kg/min), control animals received saline instead of lidocaine. Data collected at predetermined time points during the 60-min surgery included heart rate, mean arterial blood pressure, pO2, and pCO2. Cardiopulmonary variables did not differ between groups. The findings in the publication indicate that, at the dose provided intravenous lidocaine did not cause adverse cardiopulmonary effects in adult goats undergoing abdominal surgery. Adding lidocaine during general anesthesia is an option for enhancing transoperative analgesia in goats.

QUESTIONS

1.  Pain in goats may manifest through

a. Increased vocalization

b. Tachycardia

c. Tachypnea

d. Hypertension

e. Inappetence

f. Bruxism

g. Immobility

h. Abnormal gait

i. All of the above

2. T/F. The primary mechanism of action for local anesthetics is sodium channel inhibition, thus preventing nerve conduction in A and C fibers.

3.  The analgesic effects of intravenous lidocaine may be due to

a. Suppression of tonic neural discharges in injured tissue

b. A direct action on spinal transmission

c. Both A and B

4.  Lidocaine is used as

a. Local anesthetics

b. Antiarrhytmatic agent

c. Antioxidants

d. All of the above

5. T/F. Lidocaine has a reputation of increased toxicity in goats. This reputation is probably due to the common clinical use of lidocaine in animals of low body mass, such as when injecting lidocaine into the horn bud of a kid goat.

ANSWERS

1. i

2. True

3.  c

4.  d

5. True

**Carlson et al. Pharmacokinetics of 2 Formulations of Transdermal Fentanyl in Cynomologus Macaques (*Macaca fascicularis*), pp. 436-442**

Domain 3: Management of Pain and Distress

Primary Species: Macaques (Macaca spp.)

SUMMARY: An evaluation of a basic noncompartmental pharmacokinetic profile of the 25µg/h fentanyl transdermal patch and the transdermal fentanyl solution (Recuvyra®; 2.6 mg/kg or 1.95 mg/kg) in cynomolgus macaques is reported. Of note, the 2.6 mg/kg transdermal fentanyl solution resulted in severe adverse events, characterized by respiratory depression, hypothermia, bradycardia, and unresponsiveness, which were successfully reversed with naloxone and supportive care. The 1.95 mg/kg transdermal fentanyl solution dose did not result in adverse events but it negated use of the manufacturer’s applicator, limiting its efficiency. No skin irritation was noted after application of either sustained-release delivery method. Significant interanimal variability existed with both delivery methods, be it as zero concentrations throughout the study period or outlier concentrations up to120x the other study animals. Overall, higher and more protracted fentanyl concentrations were present with administration of the transdermal fentanyl solution versus the fentanyl patch. Compared to canine literature, the cynomolgus macaques had 28-300 fold higher serum fentanyl concentrations. Without further study, these sustained-release fentanyl formulations cannot be practically and effectively utilized for pain management in cynomolgus macaques.

QUESTIONS

1.  Name the neuroleptanalgesic which contains fentanyl and fluanisone.

2.  Name the neuroleptanalgesic which contains fentanyl and droperidol.

3. What is the Controlled Substances Act (CSA) Schedule of fentanyl? Define that CSA schedule.

4.  Name the adverse event reported in mice anesthetized with a combination of fentanyl and medetomidine.

5. A tiletamine/zolazepam combination was used for sedation in this study. Name the species in which tiletamine is nephrotoxic.

6.  What is the definition of a painful procedure according to the Animal Welfare Regulations?

7.  T/F. Cefovecin can is commonly used as a long-acting antibiotic in cynomolgus macaques.

ANSWERS

1.  Hypnorm

2. Innovar-Vet

3.  CSA Schedule II. A CSA Schedule II drug has high abuse potential, potentially leading to severe psychological or physical dependence and is considered dangerous.

4.   Urinary retention

5.  Oryctolagus cuniculi

6.   A painful procedure is one that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which the procedure is applied, that is, pain in excess of that caused by injections or other minor procedures.

7.  False (See JAALAS 49:805-8, 2010)

***Experimental Use***

**Dudley et al. Effects of Topical Anesthetics on Behavior, Plasma Corticosterone, and Blood Glucose Levels after Tail Biopsy of C57BL/6NHSD Mice (*Mus musculus*), pp. 443-450**

Domain 3

Primary Species: Mouse (Mus musculus)

SUMMARY:For transgenic mice to be bred for and used in research, the genotype of the individual mouse must be known. Biopsy of the distal tail tip is the most common method of obtaining genetic material for this purpose.

Previous studies in mice (age, 17 d to adult) have documented acute behavioral and physiologic responses of mice to tail biopsy which indicate that the procedure is stressful and may be painful, although the intensity of pain is unknown.

Optimally, mice undergoing tail biopsy should be afforded pain relief that does not increase the overall stress of the procedure.

This study evaluated the ability of topical anesthetics to reduce pain and stress of tail biopsy without causing physical trauma or long-term effects.

In the current study, the effects of topical 2.5% lidocaine–2.5% prilocaine (LP) cream applied to the distal tail tip at 5 or 60 min before biopsy and of immersion of the tail tip for 10 s in ice-cold 70% ethanol prior to biopsy or in 0.5% bupivacaine for 30 s after biopsy were evaluated in preweanling mice of 3 different age groups. The authors hypothesized that these treatments would reduce behavioral and physiologic responses and that younger mice would have fewer pain responses to tail biopsy than would their older counterparts.

Each group contained 10 mice (5 female, 5 male) C57Bl/6NHsd. Tail biopsies were collected when mice were 7, 11, or 15 d of age in groups designated for biopsy. Acute behavioral responses, plasma corticosterone, and blood glucose were measured after biopsy, and body weight and performance in elevated plus maze and open-field tests after weaning.

Regardless of age, mice treated with ice-cold ethanol before biopsy were 85% less likely to vocalize (*P* = 0.015) and 80% less likely to move during biopsy (*P* = 0.005) than were mice that did not receive anesthesia. This decrease in behaviors was the best evidence that dipping the tail tip in ice-cold ethanol was most efficacious at reducing the acute pain of tail biopsy. Both ice-cold ethanol and bupivacaine prevented elevations in corticosterone and blood glucose after biopsy.

Our findings that ice cold ethanol and bupivacaine prevent corticosterone elevations after tail biopsy strongly support the probability that tail biopsy in preweanling mice is a painful procedure.

Dipping the tail tip in ice-cold ethanol prior to biopsy or in bupivacaine after biopsy were the only treatments evaluated that prevented elevations in blood glucose. Tail biopsy with or without anesthesia did not affect body weight or performance on elevated plus maze or open-field tests.

The authors recommend the use of ice-cold ethanol prior to tail biopsy in mice 7 through 15 d of age.

QUESTIONS (True or False)

1. Mineralized bone is innervated with a rich network of sensory and sympathetic fibers, which allow nociception to readily occur.

2. This study’s finding that 7-d-old mice vocalized significantly more often than did 11-d-old or 15-d-old mice at the time of tail biopsy supports the possibility of pain perception as young as 7 d.

3. During the perinatal period, rodents experience increased sensitivity of the HPA axis to even mild stressors.

ANSWERS

1. True

2. True. Neonatal hypersensitivity is recognized in human infants, and a potential physiologic basis has been identified in mice. The increased vocalizations at the time of biopsy in 7-d-old mice may represent hypersensitivity to pain at this age, although further investigations are warranted.

3. False. Reduced sensitivity. In mice, this stress-hyporesponsive period occurs from birth to approximately day 12 and is characterized by very low basal levels of corticosterone and a failure to release corticosterone in response to stressors.

**Boivin et al. Physiological, Behavioral, and Histological Responses of Male C57BL/6N Mice to Different CO2 Chamber Replacement Rates, pp. 451-461**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (Mus musculus)

SUMMARY: The most recent *AVMA Guidelines for the Euthanasia of Animals* recommends a gradual displacement rate of 10% to 30% of CO2 for euthanasia in rodents. This recommendation was determined to be better for the welfare of the animal, and was based on studies of humans with nasal CO2 exposure. This study aims to reexamine the optimal CO2 replacement rate for rodent euthanasia.

C57BL/6N male mice were used for this study. Radiotelemetry devices were implanted surgically and mice were allowed to recover for 1 week. Baseline telemetry readings were taken without anyone in the room. On the day of the study, telemetry was turned on and recordings were made for 30 min prior to CO2 euthanasia. All animals were euthanized in their home cage to minimize stress. Heart rate, blood pressure, and activity were measured during the euthanasia procedure. Four different replacement rates of CO2 were tested: 15%, 30%, 50% and 100% of the chamber volume per minute. Sham procedures in which compressed air was forced into the euthanasia chamber at 15%, 30% and 50% replacement rates was used as a control. After mice were euthanized, blood was collected and tested for corticosterone and ACTH. Lungs were also collected and examined histologically for lesions.

Results: Overall, CO2 euthanasia increased cardiovascular parameters and activity in all groups. All physiological responses occurred more rapidly with a higher CO2 replacement rate. Activity levels, behavioral responses, ACTH and corticosterone levels and lung pathology were not different between groups. No evidence was found that suggests that 15% or 30% replacement rates are less painful or distressful than 50% or 100%. The authors conclude that 50% to 100% CO2 replacement rate is acceptable for euthanizing adult male C57BL/6N mice.

QUESTIONS

1. What is the current recommended CO2 replacement rate (in percent of volume of the chamber per minute) for euthanasia of rodents as outlined by the *AVMA Guidelines for the Euthanasia of Animals*?

a.  3-5%

b.  5-15-%

c. 10-20%

d.  10-30%

e.   15-25%

2. What are the authors recommendations regarding CO2 euthanasia in adult male C57BL/6N mice?

a. CO2 euthanasia is unacceptable at any replacement rate as it is too distressful for mice.

b. Higher CO2 replacement rates such as 50% and 100% are acceptable for euthanizing adult male C57BL/6N mice

c. The current AVMA guidelines are correct in that lower CO2 replacement rates appear to be less distressful for mice.

d. No conclusions could be drawn from this study

ANSWERS

1. d

2. b

**Alvarado et al. Retrospective Evaluation of Nail Trimming as a Conservative Treatment for Ulcerative Dermatitis in Laboratory Mice, pp. 462-466**

Domain 1

Primary Species: Mouse (Mus musculus)

SUMMARY: This paper takes a retrospective look at the efficacy of nail trimming in treating ulcerative dermatitis (which affects B6 mice and those with a B6 background).  Affected mice were treated in one of several ways as follows: only nail trim of hind limbs (repeated at discretion of veterinarian); oral antibiotics only (Baytril in water) in which water replaced q 5 days until resolution or euthanasia; topical antibiotics only (triple antibiotic ointment) applied SID until resolution or euthanasia; no treatment.

A veterinarian assessed the mice to determine if resolution had occurred. Resolution was achieved in 74 of 125 nail trim cases, 3 of 23 oral antibiotic cases, 3 of 12 topical antibiotic cases, and 10 of 74 observation cases.

The mice which received the nail trimming had the greatest incidence of resolution coupled with the shortest resolution time such that nail trimming by itself was found to be an effective treatment option for UD.

QUESTIONS (True/False)

1.  UD is considered to be idiopathic.

2.  Given the evidence that changes in the microbiota can affect phenotypes, one must consider the potential confound regarding the use of antibiotics in research animals.

ANSWERS

1. True

2.  True

**Redelsperger et al. Stability of Doxycycline in Feed and Water and Minimal Effective Doses in Tetracycline-Inducible Systems, pp. 467-474**

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

Primary Species: Mouse (Mus musculus)

SUMMARY: The study was conducted to assess the stability of doxycycline when incorporated in feed and water for tetracycline-inducible rodent models as well as its most effective route of administration and dose.

WATER: Concentrations of doxycycline in reverse-osmosis–purified (RO; pH 6.0) and acidified RO (pH 2.6) water in un-tinted or green-tinted bottles was assessed. Doxycycline remained stable in all groups for 7 days and in acidified water in un-tinted bottles for 14 days. Fungal growth was further seen in non-acidified water in both tinted and un-tinted bottles by 12 and 14 days, respectively, and also in tinted bottles containing acidified water on day 14, but was not seen in un-tinted bottles with acidified water.

FEEDS: Doxycycline concentrations was also assessed before and at various points after the pelleting of feed from 2 vendors. Each batch was divided for storage at 4 °C, at room temperature, or within ventilated mouse isolator cages and was then sampled monthly for a period of 6 months. It was determined that drying of feeds caused the greatest decline in doxycycline concentration, while γ-irradiation plus shipping and storage condition had minimal effect.

EXPERIMENT: Two mouse lines with tetracycline-inducible promoters received 25, 150, or 467 μg/mL or 2 mg/mL doxycycline in water and 200 or 625 ppm in feed before analyzing their GFP expression from Day0-13. Baseline blood was previously collected and blood was collected on experimental days 2, 6, and 13 for analysis.  GFP was expressed in Rosa-rtTA2 mice at a dose of 150 μg/mL, whereas Cags-rtTA3 mice required only 25 μg/mL for expression.

RESULT: The result of the study indicates that:

1.  Doxycycline-compounded feed can be handled in the same manner as standard rodent feed without decline in doxycycline concentration.

2.  Tinted water bottles are not necessary for maintaining drug concentrations

3.  Concentrations lower than those used typically may be effective in some mice lines with tetracycline-inducible promoters.

QUESTIONS

1.   Why is the use of doxycycline more preferred than tetracycline as an inducer of GM tet-dependent mice?

a.  Doxycycline has higher potency than tetracycline

b.   Doxycycline has superior tissue penetration than tetracycline

c.  Doxycycline has widespread availability

d.  All of the above

2.   True or false: Doxycycline has other biologic effects that extends beyond its antimicrobial activity

3.   True or false: Tetracyclines in general absorb UV light and is considered light sensitive and unstable in water.

ANSWERS

**1.  d**

**2.  True. Doxycycline at a certain doses may inhibit tumor cell proliferation or can reduce size of abdominal aneurysm.**

3.   **True**

**Rajaei et al. Intraocular Pressure, Tear Production, and Ocular Echobiometry in Guinea Pigs (*Cavia porcellus*), pp. 475-479**

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

T3: Diagnose disease or condition as appropriate

Secondary Species: Guinea Pigs (Cavia porcellus)

One-Line Summary: The study determined and reported on the intraocular pressures (IOP) and tear production values in guinea pigs using rebound tonometry, EAPTT and PRTT.

SUMMARY:The intraocular pressures (IOP), tear production and the effects of time of day on both parameters were measured in 24 healthy adult guinea pigs (male and female) of different breeds (American, Teddy and Dunkin-Hartley). The IOP was measured using rebound tonometry while the tear production measurements were collected using the Endodontic Absorbent Paper Point Tear Test (EAPTT) and the Phenol Red Thread Test (PRTT).  These parameters were measured at three (3) time points (0700, 1500, and 2300) and with gentle manual restraint only.  The animals’ eyes were also assessed using B-mode Ultrasonography.

Overall values were: IOP, 6.81 ± 1.41 mmHg (range, 4.83 to 8.50); PRTT, 14.33 ± 1.35 mm (range, 12.50 to 16.83); and EAPTT, 8.54 ± 1.08 mm (range, 7.17 to 10.0 mm).  From this study the found that the IOPs increased from mornings to nights with the highest recorded IOP at 2300.  The tear production results did not differ significantly between male and female guinea pigs.  No abnormal opacities seen using ultrasonography.

QUESTIONS

1. What human ocular conditions is the Guinea pig a good model for?

2.  True/ False.  The retinal vasculature of the guinea pig is holangiotic.

3.  What ocular anatomic features are shared by chinchillas and guinea pigs?

4.  What is the advantage of the Phenol Red Thread Test over the traditional Schirmer Tear test?

ANSWERS

1.  Cataracts, Human Myopia, Retinal Research

2.  False.  It is paurangiotic.

3.  They have small eyes with thick lens.

4.  The advantages include: shorter duration, the cotton thread used is less irritating to the corneal surface.

**CASE REPORT**

**Steiner et al. Use of Ronidazole and Limited Culling To Eliminate *Tritrichomonas muris* from Laboratory Mice, pp. 480-483**

Domain 1; T4 - Treatment of Disease Condition, Management of Spontaneous Disease

Primary Species: Mouse (Mus Musculus)

SUMMARY: *Tritrichomonas muris* is a gastrointestinal parasite that is occasionally detected in laboratory animal mouse populations.  Immunocompromised mice may demonstrate gastrointestinal disease with infection, but most mice do not demonstrate clinical infection.  *T. muris* is considered highly transmissible and culling or rederivation through embryo transfer is recommended to eradicate the organism from the colony.  The antiparasitic agent ronidazole has been used for effective treatment and elimination of a similar organism that infects cats, *Tritrichomonas fetus*, and was proposed to be used to help eradicate *T. muris* in mouse populations in this study.  *T. muris* was identified by examination of fecal smears and confirmed via real-time PCR assay in this particular case report.  Ronidazole was delivered in drinking water to the mice for 15 days, and mice retested.  Positive mice were culled from the colony with the exception of a breeding pair.  Additional doses of ronidazole were delivered in dextrose-spiked drinking water for an additional 42 days and mice retested.  Negative samples were obtained at 3 months and 1 year following second administration.  Ronidazole appears to be an effective treatment for T. muris infection of mice.  The use of ronidazole with separation and culling of consistently positive animals was effective for elimination of *T. muris* from the colony of immunocompromised mice in this case report without adverse effects on breeding fecundity.

QUESTIONS

1.   Clinical disease in mice infected with *Tritrichomonas muris* presents as:

a.  Diarrhea in immunocompromised mice

b.  Neurological symptoms in all strains of mice

c.   Sudden death in immunocompetent mice

d.  There are no signs of clinical disease in mice

2. *Tritrichomonas fetus* infection:

a.   Is an innocuous protozoal parasite of rodents, ruminants, and cats

b.  Is endemic in immunocompromised mouse colonies of the southwest

c.  Affects reproductive performance in cattle and cats

d.  Has no effective treatment options in any species

ANSWERS

1. a

2.  c