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**ORIGINAL RESEARCH**

***Reproduction***

**Li et al. Effects of Intrauterine Air Bubbles on Embryonic Development in Mice, pp. 7-15**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: During murine embryo transfer, air bubbles frequently are loaded with embryos into the transfer catheter. Air bubbles reportedly can induce decidualization in mice, rats, and hamsters. The authors sought to clarify if this can disturb decidual gene expression, implantation, or embryonic development.

The study was carried out using CD1 females (age, 8 to 12 wk) and males (age, 3 to 10 mo) mice housed in standard conditions.

1. Air-Induced Decidualization In Pseudopregnant Mice

On day 3.5 of pseudopregnancy (induced by making with vasectomized males), between 1500 to 1700\*, air was infused into the lumen of a single uterine horn (stimulated) by using transcervical embryo transfer (TCET) devices.

1. Air-Induced Decidualization In Pregnant Mice

On the afternoon of days 1.5, 2.5, 3.5, and 4.5, pregnant females underwent air infusion into a unilateral uterine horn by using TCET devices; in contralateral (control) horns, normal embryo implantation was allowed to occur.

1. Tetraploid Embryo-Induced Decidualization

Tetraploid embryos were prepared as described in Kaufman MH, Webb S. 1990 Development 110:1121–1132.

The tetraploid embryos were surgically transferred into the right oviducts of pseudopregnant day 0.5 mice. After 3 d, these mice underwent intrauterine air infusion into the left uterine horns.

1. Oocyte-induced decidualization

Germinal vesicle (GV) oocytes were collected from females and transferred (12 GV oocytes per recipient) into a single uterine horn of day 3.5 pseudopregnant mice by using TCET devices.

1. Embryo transfer

Naturally matured blastocysts (6 to 10; with or without air) were transferred into a single uterine horn in day 2.5 pseudopregnant mice by using TCET devices. Pregnant recipients were necropsied on day 7.5, and embryo implantation and uterine decidualization were examined. Tetraploid embryos were surgically transferred into the oviducts of day 0.5 pseudopregnant female mice.

After 3 d, these mice again underwent intrauterine air infusion into the left uterine horn by using a glass transfer pipette.

RESULTS

Air-Induced Decidualization In Pseudopregnant Mice: To choose an appropriate volume of air for intrauterine infusion in this study, the authors used TCET devices to infuse 0.5, 1.0, 2.0, 5.0, or 10.0 µL into the uterine lumen of 8 day 3.5 pseudopregnant mice. At 4 d after air infusion, all mice displayed a dilated and nonbeaded appearance, consistent with decidualization, in the infused uterine horn. This phenomenon did not occur in the contralateral uterine horn, indicating that decidualization was induced by the infused air. The mean weight of the uterine horns infused with air (725 ± 170 mg) was significantly (*P* < 0.001) greater than noninfused horns (74 ± 37mg, *n* = 8); the mean diameter of air-infused horns was greater (*P* < 0.001) as well (5.8 ± 1.2mm compared with 1.3 ± 0.7mm, *n* = 8). Decidualization induced by different volumes of infused air yielded different scores.

At volumes of 2.0 µL and lower, the score was positively correlated (r = 0.99, *P* < 0.001) with the volume of air infused; at volumes above 2.0 µL, the score did not increase with the increase in air volume. In light of these data, the authors selected a dose of 2.0 µL of air for all subsequent experiments.

Decidualization did not occur when air was infused into a single uterine lumen of day 2.5 (n = 6) or day 1.5 (n = 6) pseudopregnant mice.

3 pseudopregnant mice were euthanized at day 3.5 immediately after air infusion and isolated the uterine tissues. Stereomicroscopy revealed uterine lumens that were filled with large bubbles. In addition, the uterine horns of 3 day 3.5 pseudopregnant mice killed at 2h after air infusion contained microair bubbles in the uterine lumens. Additionally, they performed a similar operation in 3 females at the same stage, but injecting with 0.1 mL of 1% trypan blue in saline, aiming at finding out whether intrauterine air bubbles increased endometrial capillary permeability. After 3 min, the air-infused uterine horns of the 3 mice displayed a diffuse blue area. In contrast, the natural day 4.5 pregnant horn displayed distinct and isolated blue bands after trypan blue injection

Effects Of Intrauterine Air Infusion On Fetal Development In Naturally Pregnant Mice: Air (2.0 µL) was infused into a single uterine lumen (*n* = 12) of day 1.5, 2.5, 3.5, and 4.5 pregnant mice. On day 7.5, the uterine tissues of 4 females in each group were collected; the number of live pups and birth weight were recorded at delivery in the remaining dams.

Dams infused when 3.5 or 4.5 d pregnant had fewer (*P*< 0.01) live pups than those infused on day 1.5 or 2.5, but birth weight did not differ significantly among the 4 groups. The left and right uterine horns of the 16 day 7.5 females displayed visible morphologic differences.

In mice infused on day 1.5 or 2.5 of pregnancy, the conceptuses in the air-infused horns were crowded around and unevenly distributed along the uterine lumens compared with the noninfused horns. In dams infused on day 3.5 or 4.5, the air-infused horns displayed similar decidualization, comprising a dilated and nonbeaded appearance, as the air-induced decidualization of pseudopregnant mice.

The conceptuses from the air-infused horns of dams infused on day 3.5 or 4.5 had lost all anatomic structure, indicating that they were dead.

Intrauterine Air Infusion Disrupt Development Of Transferred Embryos: Blastocysts with air (2.0 µL; *n* = 48) and 32 blastocysts without air were nonsurgically transferred unilaterally into day 2.5 pseudopregnant mice (*n* = 6 and 4, respectively). On day 7.5, 10 mice from each group were killed and uterine decidualization and conceptuses were evaluated. In the nonair group, the unilateral uterine horns of all 4 mice displayed even embryo distribution (total, 24 implantation sites) with a beaded appearance, and the conceptuses in each decidua displayed normal embryonic structure. By contrast, in the air group, all 6 mice displayed uneven decidualization, with the air-infused uterine horn showing a dilated and nonbeaded appearance and containing growth-delayed or dead conceptuses.

Oocyte- And Tetraploid Embryo-Induced Decidualization: On day 7.5, the left uterine horns of all 3 mice displayed dilated and nonbeaded decidualization, whereas the right uterine horns displayed normal and beaded implantation sites. The oocyte-induced decidual responses were morphologically the same as those after air induction, whereas tetraploid-induced decidual responses were the same as those for embryo induction.

Genes Differentially Expressed Among Air-Induced And Tetraploid-Induced Deciduoma And Embryo-Induced Decidua: To explore the potential effect of the air on uterine gene expression during decidualization, the authors compared the global mRNA levels among RNA samples from day 7.5 air-induced and tetraploidinduced deciduoma and embryo-induced decidua.

Thirty-three genes were upregulated in the embryo-induced decidua compared with the air- and tetraploid-induced deciduoma. No gene differed significantly in expression between air- and tetraploid-induced deciduoma.

GO Enrichment Of Differentially Expressed Genes: Regarding upregulated genes, 16 GO terms were significantly enriched in the biologic process category. In the molecular function category, transcripts associated with hormone activity were enriched. The over-represented GO terms under the cellular component category were representative of the extracellular region and extracellular space. In contrast, none of these GO terms in these 3 categories were enriched among downregulated genes.

DISCUSSION

This study provided strong evidence that intrauterine air disrupts embryo spacing, induces deciduoma, and impairs postimplantation embryonic development. The gene expression profile of air-induced deciduoma was significantly different from that of natural embryo-induced decidua but similar to tetraploid-induced deciduoma. The data indicated that intrauterine infusion of air into the uterine horns of pregnant mice probably disrupts the process of embryo-induced decidualization.

Infused air was split into numerous micro air bubbles due to uterine contraction. These microbubbles probably adhered to embryos and endometria and then stimulated abnormal decidualization. The resulting tissues resembled deciduoma rather than decidua in morphology and gene expression profiling.

Although less than 1.0 µL of air typically is transferred with embryos into murine uterine horns, trace air potentially may significantly disrupt embryonic development, and these data suggested that air should be excluded from the catheter during murine embryo transfer.

In the current study, air-induced deciduoma usually displayed a sausage-like appearance when more than 2 µL of air was infused. However, in pilot studies, when infused air was less than 1 µL, the deciduoma usually displayed uneven beads (data not shown).

Air- and oocyte-induced decidual responses were similar in morphology, whereas tetraploid- and embryo-induced decidual responses resembled each other morphologically, and tetraploid induction was completely indistinguishable from pregnant decidualization in morphology. This tetraploid model of decidualization likely is more ‘physiologic’ than other artificial methods of inducing decidualization. gene expression profiling in tetraploid-induced decidualization differed significantly different from naturally pregnant decidualization. The gene expression profile of tetraploid-induced deciduoma was significantly different from that of natural embryo-induced decidua and was almost identical to that of air-induced deciduoma.

Consequently, the tissues resulting from tetraploid induction technically are deciduoma instead of decidua.

In light of the current study, the authors suggest that the traditional view of an antagonistic relationship between the conceptus and the uterus should be revised; instead, an ICM-directed decidualization model is perhaps more appropriate. A relay mechanism exists among the ICM, trophectoderm, uterine endometria, and other target cells. As an intermediate, the trophectoderm relays ICM-derived signals to other target cells. If the ICM is not developing well, the trophectoderm relays ‘no/wrong’ signals regarding decidualization, and pregnancy failure would result.

\* Note: measurement unit is not indicated in the paper

QUESTIONS

1. T/F. Decidualization can happen without the presence of an implanting embryo, by physical stimulus as small beads or droplets injected into the uterine lumen.

2. T/F. Infused air volumes below 1 ul do not cause any effect

3. The most severe disruption of air infused at blastocyst transfer happens at:

* 1. In 2.5 days recipients
	2. In 3.5 days recipients
	3. Both 2.5 and 3.5 display similar disruption
	4. No disruption is observed

ANSWERS

1. T

2. F

3. b

***Husbandry***

**Winn et al.** [**Daily Water Intake by Common Marmosets (*Callithrix jacchus*) and Recommendations Regarding Fluid Regulation**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00002)**, pp. 16-20**

Domain 3: Research

Secondary Species: Marmoset/Tamarin (Callitrichidae)

SUMMARY: In order to improve task consistency in macaques, some form of fluid regulation is often used. This controlled water access is calculated off of baseline water consumption of the species, which does not currently exist for the common marmoset. Twenty-two marmosets were split into 4 groups: older males, older females, younger males, and younger females, some single housed and some pair housed. They were allowed to drink from water bottles that were weighed at various time points over a total of 27 nonconsecutive days in order to calculate water intake. Overall daily water intake was found to be 61.3 +/- 20.4ml/kg. Water intake was consistent across single and pair housed animals, was not significantly correlated with BCS, and there was no significant difference in consumption based on weekday vs. weekend. Daily intake was higher for older marmosets than younger ones and a significantly larger amount of water was consumed when room lights were on vs. when off. When biscuits were soaked in water before feeding to increase moisture, marmosets drank significantly less water. Given these findings, fluid regulation protocols can be developed for this species in conversations with veterinary staff, lab staff, and the IACUC.

QUESTIONS

1.   True or False: Natural social structure in marmosets is fission-fusion.

2.   True or False: The marmoset is often used as a model for ulcerative colitis.

3.  The scientific name of the common marmoset is:

a.  *Saguinous oedipus*

b.  *Callithrix jacchus*

c.  *Leontopithecus rosalia*

d.  *Nycticebus coucang*

ANSWERS

1.     False

2.     False

3.     b

***Anesthesia***

**Gibbs et al. Effects of General Anesthesia on 2 Urinary Biomarkers of Kidney Injury—Hepatitis A Virus Cellular Receptor 1 and Lipocalin 2—in Male C57BL/6J Mice, pp. 21-29**

Domain 3: Research;  T3: Design and conduct of research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Urinary biomarkers are used to predicted kidney injury in preclinical and clinical studies. The purpose of this study was to determine the effect of five anesthetic regimens (ketamine-xylazine, tiletamine-zolazepam, pentobarbital, isoflurane, sevoflurane) on kidney injury biomarkers. The two biomarkers studied were hepatitis A virus cellular receptor 1 (HAVCR1 or KIM1), a marker that is absent in normal urine but upregulated in injured proximal tubular epithelial cells and 2(2) Lipocalin 2 (LCN2) which is normally reabsorbed in the proximal tubule, so the protein and gene expression are rapidly increased after injury to epithelial cells, including renal tubular epithelia. Animals showed increased HAVCR1 levels at 6h and decreased LCN2 levels with ketamine-xylazine anesthesia. No other groups showed significant changes in HAVCR1 relative to baseline and the ketamine-xylazine group normalized at 12h. LCN2 levels increased over 24h following inhalant anesthesia (significant in sevoflurane) but decreased in all injectable groups. Renal histology scores did not differ significantly among groups and no inflammatory cells were present in urinary bladder sections. While none of the studied anesthetic regimens alone are likely to cause AKI, renal injury is additive, so combination with other nephrotoxic agents (e.g. NSAIDs) may have different results.

QUESTIONS

1.  HAVCR1 is significantly increased in urine at 6h but returned to baseline at 12h with which of the following anesthetic regimens?

a.  Isoflurane

b.  Sevoflurane

c.  Pentobarbital

d.   Ketamine-xylazine

e.  Tiletamine-zolazepam

2.   Ture or False. Ketamine-xylazine was found to have lasting renal effects that may confound studied interested in markers of AKI.

a.     True

b.     False

ANSWERS

1. d
2. False

**Erickson et al.** [**Alfaxalone–Xylazine Anesthesia in Laboratory Mice (*Mus musculus*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00004)**,** **pp. 30-39**

Domain 2

Primary Species – Mouse (*Mus musculus*)

SUMMARY:  Isoflurane is one of the most commonly used and preferred methods of general anesthesia in mice.  However, it is not always practical or possible to use this inhalant form of anesthesia.  The most common method of injectable (bolus) anesthesia in mice is ketamine/xylazine, but this regimen can have unpredictable effects.  This paper examined the combination of the neuroactive steroid Alfaxalone and Xylazine as an alternative injectable anesthetic.  Dose ranges, physiological parameters, were measured in B6 and CD1 mice (males and females).  The three doses that were used were 40, 80, and 120 mg/kg Alfaxalone combined with 10 mg/kg Xylazine.  Heart rate, respiratory rate, and temperature were measured after loss or righting reflex (LORR).   Loss of pedal withdrawal reflex (PWR) was considered a surgical plane of anesthesia.  A laparotomy was also performed on the group receiving 80 mg/kg Alfaxalone.  Orthopedic surgery with Alfaxalone was also performed with Isoflurane as a control.

Mortality rates for mice undergoing laparotomy procedure was elevated when the anesthetic was delivered IP, but SC delivery provided better results.  Alfaxalone/Xylazine IP worked well for orthopedic procedures and should work well for minor procedures.  There were significant sex and strain differences including female CD1 mice being less sensitive to the cocktail than the female B6 mice.  Heart rate for the B6 mice was also dramatically lower than the CD1 mice, which appeared to have almost no change at all from baseline.  The authors recommend using Atipamezole for reversal in order to speed up recover time and to reduce the rates of mortality.

QUESTIONS

1.  Alfaxalone is a neuroactive steroid that serves as a:

a.  GABA antagonist

b.  GABA agonist

c.  Alpha 2 antagonist

d.   Alpha 2 agonist

2. In this paper the laparotomy procedure in two groups of mice (B6 and CD1) under a cocktail of Alfaxalone/Xylazine.  What was recommended as the route of administration for lower levels of mortality?

a.  IP

b. IV

c. PO

d.   SC

3. When used as a general anesthetic in mice, what is the purpose of adding Xylazine in combination with Alfaxalone?

a.  Smoother induction

b.   Decreased myoclonic activity

c. Minimized intraperitoneal irritation

d.   Reduced seizure activity

ANSWERS:

1.  b

2. d

3.  b

**Jiron et al.** [**Comparison of Isoflurane, Ketamine–Dexmedetomidine, and Ketamine–Xylazine for General Anesthesia during Oral Procedures in Rice Rats (*Oryzomys palustris*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00005)**,** **pp. 40-49**

Domain 3: Research

Tertiary Species: Other Rodents

SUMMARY: Rice rats are a well-established model for periodontitis and osteonecrosis of the jaw, and may require surgical procedures within the oral cavity as part of the experimental plan. However, as rice rats are an emerging animal model, there is a paucity of data outlining optimal anesthetic protocols for this species. The overall purpose of this study was to compare three anesthetic protocols: inhaled isoflurane via a nose cone, ketamine-dexmedetomidine (50mg/kg IP, 0.125mg/kg IP) and ketamine-xylazine (50mg/kg IP, 4 mg/kg IP) and determine which protocol(s) consistently provide a surgical level of anesthesia for approximately 30 minutes while allowing full access to the oral cavity. 22 rice rats were anesthetized with all three anesthetic protocols with a 7 day “wash out” period between each anesthetic procedure. The study found that isoflurane administered via a nasal cone,  was the most reliable and effective anesthetic for maintaining a surgical plane of anesthesia for 30 minutes, had a shorter period of induction, and a faster recovery compared to both injectable forms of anesthesia.

QUESTION

1. In mice, isoflurane exerts \_\_\_\_\_ cardiodepressive effects compared to injectable agents, such as pentobarbital, urethane, and ketamine-containing combinations

a. Greater

b. Lesser

2. What is the dental formula for rats?

ANSWERS

1. b

2.   (I 1/1, C 0/0, PM 0/0, M3/3)

**Browning et al.** [**Comparison of Dexmedetomidine–Ketamine–Midazolam and Isoflurane for Anesthesia of Black-tailed Prairie Dogs (*Cynomys ludovicianus*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00006)**,** **pp. 50-57**

Primary Species: Other Rodents

Domain 2; T2

SUMMARY: Prairie dogs have been used in research as a model for gallstone formation, infectious and zoonotic diseases, and they are a keystone species in prairie ecology.  Inhalant anesthetic agents, such as Isoflurane, are commonly recommended for handling of fractious animals.  Injectable anesthesia is less studied in prairie dogs and this study’s objective was to compare physiologic effects of inhalant anesthesia versus injectable combination of dexmedetomidine, ketamine, and midazolam (DKM).  A random crossover design was used where the prairie dogs were anesthetized twice with a 3-day washout period prior to the second anesthetic event.  Both protocols provided safe and effective anesthesia.  Dexmedetomidine is an α2 agonist that provides analgesia, sedation, and muscle relaxation and is reversible.  Ketamine is a dissociative, centrally acting NMDA receptor antagonist, which also provides analgesia.  Midazolam is a reversible benzodiazepine.  In this study, isoflurane had a longer induction time, while DKM has a longer recovery time.  Isoflurane provided a more stable anesthetic plane but did not provide analgesia.   Bradycardia occurred with DKM, respiratory rate remained stable, and body temperature was lower with isoflurane.  Neither protocol affected MAP, venous pCO2 was greater with DKM, and lactate was higher in the DKM group.

QUESTIONS

1. What have prairie dogs been used as a model for?
2. T/F.  Dexmedetomidine is an α2 antagonist that provides analgesia, sedation, and muscle relaxation and is irreversible.
3. T/F.  Midazolam is a reversible benzodiazepine.

ANSWERS

1. Gallstone formation, infectious and zoonotic diseases, and they are a keystone species in prairie ecology
2. False.  Dexmedetomidine is an α2 agonist that provides analgesia, sedation, and muscle relaxation and is reversible.
3. True

**Darbyshire et al.** [**Anesthesia and Euthanasia of Brine Shrimp (*Artemia franciscana*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00007)**,** **pp. 58-64**

Domain 2, Tasks 3 and 4: Administration of anesthesia and Euthanasia

Tertiary Species – Invertebrate

SUMMARY: This study evaluated three methods of anesthesia and three adjunctive methods of euthanasia for brine shrimp.  The three methods of anesthesia evaluated were 60% alcohol, 4g/L TMS (Tricaine methane sulfonate; MS 222), and 2.5 mg/L eugenol. Tank water was used as a control. The three methods of euthanasia evaluated were 70% alcohol, 95% alcohol, 10% neutral buffered formalin.  Anesthesia was considered effective when the brine shrimp stopped swimming forward and did not respond to stimulation by a paddle. Euthanasia was considered as effective when there was no movement of the thoracopod for 10 seconds.

Five brine shrimp were placed into wells containing each anesthetic solution or a control. Time to anesthesia and any changes in behavior were recorded for each anesthetic group. Next, groups of *Artemia (n=30)* were placed into each anesthetic solution and observed for 5 minutes. Following anesthesia, *Artemia* were then transferred to wells containing one of the three euthanasia solutions. A subset of *Artemia* from each anesthetic group were rinsed and placed in a well containing tank water for 2 hours to evaluate recovery. Anesthesia, euthanasia, recovery and behavior tests were completed three time on separate days.

None of the anesthetic solutions were found to be reliable; however all three euthanasia solutions were found to be effective.  Eugenol anesthesia was not repeatable. A larger dose (130mg/L) of eugenol was also evaluated and did not provide consistent anesthesia. TMS only produced anesthesia in 70% of the brine shrimp and took on average over 30 minutes. TMS was significantly more likely to result in recovery of anesthesia with 80% recovering in 2 hours.  Although 60% alcohol produced anesthesia in nearly 100% of the *Artemia* this solution was also inconsistent and produced the largest amount of behavioral abnormalities (characterized by hyperactivity, abnormal posturing, and assuming a curled position). Lower concentrations of alcohol were also evaluated. No anesthesia was observed with concentrations below 30%. When 40% alcohol was used, anesthesia occurred after 30 minutes compared to 10 minutes with 60%.

Overall, based on the results of this study, the authors do not recommend using any of the three anesthetic solutions tested. With an appropriate first -step anesthetic, however, all three euthanasia solutions could be used. More research is needed into what constitutes abnormal behavior of *Artemia* as well as the best methods for anesthesia and euthanasia.

QUESTIONS:

1. True/False. It is known that brine shrimp (and other invertebrates) possess nociceptors and are capable of sensing pain and distress.
2. True/False. A solution of 60% alcohol is the best method to use as a first-step anesthetic when euthanizing brine shrimp.
3. What does the AVMA Guidelines for the Euthanasia of Animals (2013 Edition) recommend as acceptable for euthanasia of brine shrimp?
4. What does the AVMA Guidelines for the Euthanasia of Animals (2013 Edition) consider as unacceptable methods for euthanasia of brine shrimp?
5. Removing from water and allowing to die from hypoxia or gill desiccation
6. Leaving in a container of water without adequate aeration (causing death by anoxia)
7. Any death due to caustic chemicals or traumatic injury without first inducing unconsciousness
8. All of the above

ANSWERS

1. FALSE. It is unclear whether invertebrates feel pain or distress. The presence of nociceptors alone in *Artemia* do not imply they feel pain.
2. FALSE. Despite AVMA Guidelines which recommend lower concentrations of alcohol for first-step anesthesia, the authors of this study do not recommend 60% alcohol as an anesthetic for brine shrimp. 60% alcohol provided inconsistent results and produced abnormal behaviors. Lower concentrations were even less effective.
3. The AVMA Guidelines recommend a 2-step process for aquatic invertebrates. Acceptable first-step methods listed are: eugenol (0.125mL/L), ethanol (1%-5%,but not 70% alcohol) and magnesium salts(although ineffective for crustaceans). Acceptable second-step methods listed are: 70% alcohol, neutral buffered 10% formalin, pithing, freezing, and boiling.
4. d. All methods listed are considered unacceptable

***Experimental Use***

**Liepert et al.** [**3D-printed Wash Station with Integrated Anesthesia Delivery Manifold for High-throughput Depilation of Laboratory Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00008)**, pp. 65-70**

Domain 2; T3 - Administration of Anesthesia

Primary Species: Mouse (*Mus musculus*)

SUMMARY: A group in the Freimann Life Science Center at the University of Notre Dame, Indiana, designed and fabricated a device that allows for multiple mouse anesthesia while using depilatory crème for surgical site preparation. They coined the device a Mouse Depilation Station (MDS). The MDS was designed to integrate important features to make it a robust, safe, and efficient hair-removal platform, of which includes: 1.) clean, efficient, and safe hair removal from the mouse by providing a platform to customize and batch-process mouse depilation (3 mice at a time, with ovoid, center-depressed, tilted, and slatted resting surfaces); 2.) enhanced the safety of anesthetic gas delivery to murine subjects as well as saving processing time which thereby decreased total anesthetic time necessary for surgical site preparation; and 3.) providing a waste collection system to improve cleanliness of depilation process, thereby decreasing paper refuse and researcher inconvenience. The device was manufactured using a 3D-printing device, and made of sanitizable and sturdy materials.

QUESTIONS

1. The device pictured below (C) facilitates what research practice?



1. Depilation in preparation for aseptic surgery
2. Serial Tail flick response monitoring for Pain Assessment
3. Sterile abdominal organ collection for tissue culture
4. Platform for bioluminescent imaging for tissue engraftment assessment
5. The images depicted below were obtained during a research study of prostate cancer. What agent was most likely administered and what imaging modality was used to provide the images below?

 

1. Galladinium, Radiography
2. Ferritin, Near-infrared Imaging
3. Luciferase, Bioluminescence Imaging
4. Fluorodeoxyglucose, Positron Emission Tomography
5. Classify the following viruses:
	1. Mouse Hepatitis Virus
	2. Minute Virus of Mice
	3. Ectromelia
	4. Lymphocytic choriomeningitis virus
	5. Sendai Virus

ANSWERS

1. a. This is the Mouse Depilation Station (MDS)
2. c. (Source for picture: <https://www.mibioresearch.com/imaging/bioluminescence-imaging/>)
3. a. MHV - Coronavirus

b. MVM - Parvovirus

c. Ectromelia (PoxVirus) - Orthopoxvirus

d. LCMV - Arenavirus

e. Sendai - Paramyxovirus

**Kick et al.** [**Evaluation of 4 Presurgical Skin Preparation Methods in Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00009)**, pp. 71-77**

Domain 3: Research - Knowledge required to perform tasks; K14: Aseptic requirements for performing surgery

Primary Species – Mouse (*Mus musculus*)

SUMMARY: Mice used in biomedical research frequently undergo surgical procedures as part of a research protocol. A vital component of aseptic surgery and one necessary to ensure a positive outcome is preparation of the skin prior to the procedure. Asepsis is defined as creation of a skin surface sufficiently free of gross contamination and resident microbial flora so that the risk of surgical site infection (SSI) or systemic infection is acceptably reduced. For rodents, skin preparation involves hair removal followed by cleaning of the skin with antimicrobial agents. Procedures for hair removal and skin disinfection, in many cases, are based on studies of prevention of SSI in humans, which can occur at a rate of up to 2% for ‘clean’ surgeries.  Depilatory creams are corrosive agents and may be used for hair removal in some cases. This study was undertaken to examine whether certain combinations of skin preparatory agents would contribute to an increase in skin inflammation resulting in poor imaging quality for echocardiography. Four combinations of skin preparatory methods were examined: depilation or shaving with clippers plus povidone-iodine scrub with either alcohol or sterile saline rinse. Male mice were anesthetized with isoflurane and subjected to one of the skin preparation methods.  A skin incision was made and closed using suture, and the following parameters were evaluated (from Day 0-day 7):  bacterial load, surgical wound ASEPSIS score, gross pathology, and microscopic evaluation of the wound after euthanasia. The wound scores were in this rank order on day 1: shaving, iodine + alcohol >  depilatory cream, iodine alcohol > shaving, iodine, saline > depilatory cream, iodine, saline (lowest score). Similarly, the histopathology score was shaving, iodine, saline > shaving, iodine, alcohol > depilatory cream, iodine, alcohol > depilatory cream, iodine, saline. On day 7, the wound scores were shaving, iodine, saline > depilatory cream, iodine, alcohol > depilatory cream, iodine, saline > shaving, iodine, alcohol. The histopathology score on day 7 was similar to that on day 1.  Animals that were shaved (compared to depilatory cream application) had increased follicular inflammation and epidermal damage with ulcerations, even though animals were shaved by an experienced veterinarian. Interestingly, the iodine contributed the majority of the antimicrobial effect and it appears that the alcohol had only a small impact (compared to saline) and use of depilatory cream seemed to have an antimicrobial effect.  The data indicate that all methods were effective in achieving asepsis and resulted in satisfactory wound healing by day 7.  Although histopathology revealed statistically significant differences, these were considered biologically irrelevant.

QUESTIONS

1.   Name 3 ways of specific (species) bacterial identification upon isolation from a bacterial culture. (Comment on specificity and accuracy).

2.   All of the following were considered criteria for the ASEPSIS wound score EXCEPT?

a.   Erythema

b. Quality of exudate

c.  Swelling

d.   Color (redness or pallor) of skin

e.  Tissue quality, such as separation of deep tissues

ANSWERS

1.   After isolation, the following tests can be used

* + - Biochemical testing kits (such as API strips or Microgen)
		- Molecular methods (such as PCR)
		- Mass spectrometry (MALDI-TOF analysis)
		- Old school methods of differential culture media, colony morphology, and staining (such as Gram or ACID fast) can also help in identification

Comments:  Biochemical tests and ‘old school’ methods are not always specific and do not work well for new species detected.  PCR and MALDI-TOF are the most specific.  (This paper uses MALDI-TOF).  PCR may be more costly and primers must be available for specific geneses.   Similarly, MALDI-TOF is rapid, sensitive, and economical in terms of both labor and costs involved but a limitation of the technology is that identification of new isolates is possible only if the spectral database contains peptide mass fingerprints of the type strains of specific  genera/ species/subspecies/strains.(Singhal et al., 2015, not a lab animal reference).

2.   d.  (color was not used in this case, see methods, p. 72).

BONUS!   Microbiology mnemonics.

**Gram Positive RODS only:  My BLACC List**

Mycobacterium
Bacillus anthracis

Actinomyces and Acne bacteria

Clostridium

Corynebacterium

Listeria

Also, don’t forget, morphologically, Corynebacterium look like ‘Chinese characters’

**Capsule Producing Agents (most bacteria, one fungus):**

**S**ome **S**erious**K**illers **H**ave **P**retty **N**ice **C**apsules

Strep pneumoniae, Strep agalactiae (Group B strep)

Salmonella typhi

Klebsiella pneumoniae

Haemophulus influenzae type B

Pseudomonas aeruginosa

Neisseria meningitides

Cryptococcus neoformans (fungi)

**Gram stain limitations (Gram negative or Gram variable):**

**T**hese **T**iny **R**ascals **M**ay **M**icroscopically **L**ack **C**olor

Treponema

Toxoplasma

Rickettsia

Mycoplasma

Mycobacteria

Legionella

Chlamydia

(Note, many of these target macrophages and are intracellular).

**How to differentiate between E. cuniculi and Toxoplasma in tissue:**

E. Cuniculi:  Gram +, Giemsa –

Toxoplasma:  Gram -, Giemsa +

**Hickman.** [**Wellbeing of Alcohol-preferring Rats Euthanized with Carbon Dioxide at Very Low and Low Volume Displacement Rates**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00010)**, pp. 78-82**

Domain 2; T2, T4

Primary Species - Rat (*Rattus norvegicus*)

SUMMARY: The 2013 AVMA Guidelines on Euthanasia recommends the use of 100% carbon dioxide at low (10% volume displacement/min) and very-low (30% volume displacement/min) flow rates to euthanize small rodents. The use of 70% VD/min was deemed acceptable as recently as 2000, but later revised as humans report pain at inhaling 50% CO2.

The threshold of rodents’ CO2 detection is estimated at 7-10%, and 20% induces air hunger and dyspnea. Therefore, low and very-low VD rates may be causing unnecessary distress, as they provide 1-3 minutes of CO2 exposure before loss of consciousness.

Loss of consciousness is deemed to occur around 40% in rodents. Therefore, at 70% VD/min, pain would theoretically not be experienced before the rodent is unconscious.

The author hypothesized that rats euthanized with 10% and 30% VD/min of 100% CO2 would display increased evidence of distress through behavioral and physiologic markers.

24 male and 24 female rats were euthanized with either 10% VD/min, 30% VD/min or 70% VD/min with 100% CO2. Behavioral scoring from video assessed the number of times rats reared before they went nose-down (estimate of stage II anesthesia/loss of consciousness) and the time spent rearing their head above 5 and 7 inches. After stage III anesthesia, the author terminally collected cardiac blood for blood glucose and serum corticosterone, then induced bilateral pneumothorax.

Time to nose-down was significant between all groups, with the 10% VD/min group taking the longest. The 10% group also had significantly higher levels of glucose than the other groups. The 10% group also spent a higher proportion of time standing. The mean time for rearing was longer for 10% than for other groups.

The 30%VD/min group had a lower glucose but similar corticosterone level as the 10%. The 30% group had an increased number of rears per minute compared to the 10% and 70% group.

The 70% group had the shortest time to nose down, lowest glucose, and lowest corticosterone. They spent less time rearing and less time per rear.

ACTH and noradrenaline may be better biomarkers for stress because they increase more rapidly than glucose and corticosterone.

The study suggests that 70%VD/min of 100% CO2 may be appropriate because the rats in this group displayed fewer behavioral signs of distress and had lower biomarkers of stress than did the rats in the 10% and 30%VD/min flow rate groups.

QUESTIONS

1.  At what concentration of inhaled CO2 do rodents experience unconsciousness?

a. 20%

b. 30%

c. 40%

d. 70%

2.  True or false, ACTH and noradrenaline increase more rapidly than blood glucose and serum corticosterone?

ANSWERS

1.   c

2.   True

**Soroori et al.** [**Ratio of the Bronchial Lumen to Pulmonary Artery Diameter in Rhesus Macaques (*Macaca mulatta*) without Clinical Pulmonary Disease**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000001/art00011)**, pp. 83-86**

Domain 1; Task 3 - Diagnose disease or condition as appropriate

Primary Species – Macaques (*Macaca spp.*)

SUMMARY: Chronic airway disease often results in irreversible dilation of the bronchial lumen termed bronchiectasis in humans and animals. One method to detect bronchiectasis caused by lower airway disease is to measure the diameter of the bronchial lumen and the associated pulmonary artery on CT images and determine the ratio of these two. This bronchoarterial ratio is then used as a reference to detect lower airway disease in humans and animals. The purpose of this study was to establish a reference for this ratio in healthy rhesus macaques which are used as models for human pediatric disease. Twelve (5 female, 7 male) adult (4-20 yrs; 5.0-8.6 kg body weight) clinically healthy rhesus macaques were anesthetized with medetomidine 0.05 mg/kg IM and maintained on isoflurane inhalation via endotracheal intubation. CT scans were obtained during a 60-second breath hold and repeated after contrast administration (Iohexol 3 mL/kg IV). A total of 504 ratios from 7 locations were obtained and then interpreted by a veterinary radiologist and a small animal internist. The overall bronchoarterial ratio was 0.59  0.04. There was no difference between ages or sex and no difference between lung lobes but there was a positive effect from weight. For comparison with adult humans, the ratio is 0.62  0.02. Authors noted that the bronchial tree of rhesus macaques is nearly identical to that of humans but macaques have a well-developed cardiac lobe which is absent in humans. Study limitations: small sample size; positive correlation with weight may be due to age/weight linear relationship so further study on the effect of age needs to be done; health status did not include BAL or biopsy; methods of ventilation not considered.

QUESTIONS

1. What is one of the most common sequelae of chronic airway disease?
	1. Bronchiectasis
	2. Bronchitis
	3. Bronchomalacia
	4. Bronchiolitis
2. What is the normal bronchoarterial ratio on CT in healthy macaques according to this study?
	1. 0.54
	2. 0.59
	3. 0.62
	4. 0.68
3. For which human life-stage are rhesus macaques used as models of respiratory disease?
	1. Adolescent
	2. Old-age
	3. Adult
	4. Pediatric

ANSWERS

* + - 1. a
			2. b
			3. d

**CASE REPORT**

Domain 3

Primary Species – Mouse (*Mus musculus*)

SUMMARY: This article describe the validation of an autoclave program suitable for daily use in a small rodent biocontainment unit. They evaluated several procedures for processing mouse carcasses in a standard autoclave. Heat sensors and biologic indicators were implanted inside the peritoneal cavity of dead mice, which were loaded at various densities into IVC cages or metal boxes. Heat sensors revealed broad differences in temperature inside carcasses compared with the autoclave chamber. Achieving the appropriate sterilization temperature was considerably prolonged in carcasses compared with typical laboratory waste material. They showed that for 5 cadavers placed well separated inside an IVC, a modified program for mouse cage sterilization using 134 °C for 15 min is suitable. To sterilize approximately 1 kg of carcasses in autoclavable boxes, a period of 6 h is required to reach an effective temperature of 121 °C for 60 min at the center of the waste by using an autoclave program for liquids. They validated 2 protocols for the sterilization of potentially infectious mouse carcasses, to ensure the application of efficacious procedures.

QUESTIONS

1. Achieving the appropriate sterilization temperature was considerably \_\_\_\_\_\_\_\_\_ in carcasses compared with typical laboratory waste material.

a. Shorter

b. Equal

c. Prolonged

d. None Of The Above

2. To sterilize approximately 1 kg of carcasses in autoclavable boxes, a period of \_\_\_\_\_\_\_ is required to reach an effective temperature of 121 °C for 60 min at the center of the waste by using an autoclave program for liquids.

a. 30 minutes

b. 1 hour

c. 2 hours

d. 6 hours

3. The most widely used animal species for biomedical research are \_\_\_\_\_\_\_\_\_.

a. Dog

b. Primates

c. Rats

d. Mice

e. None of the above

4. The 3 main techniques described for the decontamination of carcasses are

a. Incineration

b. Alkaline Hydrolysis

c. Rendering

d. Autoclave

5. T or F. Alkaline Hydrolysis is considered one of the biologically safest methods: the carcass is burned to ash in a controlled atmosphere; commercial units with oil or gas burners, automatic timers, and smoke discharge stacks are available.

6. T or F. Rendering describes the controlled crushing of carcass material and its subsequent heating to 115 to 145 °C for 40 to 90 min.

7. This process destroys all proteins and is suitable for the decontamination of many infectious agents, including prions.

a. Autoclave

b. Renderin

c. Incineration

d. Alkaline Hydrolysis

8. The use of \_\_\_\_\_\_\_\_\_\_\_\_\_ spores is generally recommended for biologic validation of the autoclaving of biohazardous waste

ANSWERS

1. c

2. d

3. d

4. a, b, and c

5. F ( incineration)

6. T

7. d. Alkaline hydrolysis digestion uses aqueous solutions of alkali metal hydroxides, such as NaOH or KOH. In this method, the carcass is covered with alkali solution (0.02 kg of a 50% NaOH solution for every 1 kg of carcass weight) and heated to 110 to 120 °C in a stainless steel pressure vessel for 18 h.

8. Geobacillus stearothermophilus