**ILAR J**

Volume 61, Numbers 2-3, 2020

***Marmoset: Current Topics in Research, Care and Welfare***

# **Burns and Silva.** [**Current Topics in Research, Care, and Welfare of Common Marmosets**](https://academic.oup.com/ilarjournal/advance-article/doi/10.1093/ilar/ilac001/6523265)**, pp. 107-109**

Secondary Species: Marmosets/Tamarins (Callitrichidae)

SUMMARY: The common marmoset (*Callithix jacchus*) has been maintained in captivity for biomedical research for decades.  There has been increased interest in the 21st century for their usage as an alternative NHP model.  Their small size, high fecundity, short timeline to reproductive maturity and their relatively lower occupational health risk make them an attractive model.  *Callithrix jacchus* is the most commonly used marmoset species, however there are multiple marmoset species endemic to Brazil.  Hybridization naturally occurs between the various*Callithrix*species.  Current areas of research which use marmosets include neuroscience, transgenics, obesity and metabolism, infectious disease, autoimmune disease and aging. The social and highly vocal nature of marmosets along with unique anatomic features of their brain support their use for basic neuroscience and behavioral studies.  Their small body size allow the use of rodent imaging systems such as MRI.  Some of the limitations include the lack of validated diet recommendations, nutrition and husbandry practices.  Additionally, there are few studies evaluating the efficacy and indications for commonly used anesthetic and analgesic agents in marmosets.  The creation of the Marmoset Working Group has provided a forum for collaboration and training for all users, however there is still a lack of validated diet recommendations, nutrition and husbandry practices.  No validated/standardized diet has been developed and there are great variations in definitions of underweight/overweight marmosets. There is a continued need for scientific studies to elucidate the optimal diet and husbandry to aid in the precise etiology of spontaneous gastrointestinal disease that plagues this species.

QUESTIONS

1.  T/F: Marmosets are native to Brazil where hybridization occurs among several species.

2.  T/F: Marmosets are usually fed cafeteria-style diet in biomedical research facilities.

3.  The common marmoset used in research is:

a. *Callithrix geoffroyi*

b. *Callithrix flaviceps*

c.*Callithrix jacchus*

d.*Callithrix aurita*

4. A chronic problem with marmosets is:

a.  Spontaneous gastrointestinal disease

b.  Epistaxis

c.  Encephalopathy

d.  Spontaneous blindness

5. Marmosets are used as a model in:

a. Obesity

b.  Neuroscience

c.  Behavioral studies

d.  All of the above

ANSWERS

1.  True

2.  True

3.   c

4.  a

5.  d

# **Malukiewicz** **et al. An Introduction to the *Callithrix* Genus and Overview of Recent Advances in Marmoset Research, pp. 110-138**

Domain 3; TT3.3

Secondary Species: Marmosets/Tamarins (Callitrichidae)

SUMMARY: Callithrix genus are small Brazilian monkeys composed of 6 naturally allo- and parapatric species.  C. aurita, C. flaviceps, C. geoffroyi, and C. kuhlii are endemic to the Atlantic Forest while C. jacchus can also be found in semi-arid area of northeastern Brazil (see map below).  C. penicillate has the widest geographic distribution.  At the borders of their geographical distributions, natural hybridization can occur.

C. aurita is listed as endangered.  It’s the largest species sharing morphological similarities with C. flaviceps.  C. flaviceps is listed as critically endangered.  They use a scan and pounce approach to catching small prey.  C. geoffroyi is very flexible in the types of habitats it can occupy, from semi-deciduous to deciduous forests.  They can also inhabit urban areas.  C. jacchus is the smallest species and have specialization for exudivory while the rest of the genus are primarily gummivory.  C. kuhlii’s diet is heavily fruit and nectar based.  They inhabit restinga forest, riverside forests, secondary forests, mangroves, fruit groves, and coconut and palm oil trees and are common in urban areas.  C. pencillata is flexible, inhabiting altered areas and secondary vegetation.  They can also be found in urban areas.

Marmosets are used as biomedical models for many diseases and conditions such as pediatric obesity and Zika infection.  Marmosets have delayed embryogenesis compared to humans, which is important when using marmosets to model pregnancy and development.  In marmoset social structure, generally 1 female that reproduces regardless of the number of adult females present.  Marmoset litters usually consist of twins or triplets that share a bidiscoid placenta with fused chorion and vascular anastomoses.  This can lead to an individual with more than 1 distinct genotype, or chimerism.  Blood-derived tissues are significantly more likely to be chimeric, which is why marmosets are hematopoietic chimeras.  It is possible that chimerism is a mechanism by which founder populations can quickly increase their genetic diversity and gene expression, making them more adaptable to novel environments.  Callithrix are also used in cognition studies.  They are a promising model for evolutionary and functional questions in primate personality.  And personality structure is similar between wild and captive animals.  Social dynamics require more long-term studies, but it is noted that the strongest social interactions within C jacchus are between male caregivers and infants.

Since marmoset genome is sequenced, CRISPR/Cas9 gene knockins have generated transgenic marmosets to model human disease.  Marmoset epigenetics studies have examined DNA methylation patterns and have found variation can be associated with variation in transient traits such as body weight.  Brain development, evolution, and plasticity is being explored, and some research has assayed candidate gene expression responses following severe psychological perturbations.  Marmoset cell lines that are readily used include embryonic stem cells and induced pluripotent stem cells.  Complex immunogene families such as Major Histocompatibility Complex Class I and Natural Killer Complex have been sequenced and annotated for C jacchus.

Callithrix has been a model for Epstein-Barr virus, hepatitis A infection, parainfluenza virus type 1, Flaviviridae-like virus, Oropouche virus, and Simian foamy virus.  Recently, they have been a model for arthropod borne viruses.  Wild Callithrix are exposed to arboviruses such as the flavivirus, yellow fever.  They are also susceptible to dengue virus, zika virus, Mayaro virus, and Chikungunya.  Humans can transmit human herpesvirus 1 to marmosets and is fatal.

Marmosets are also captured and trafficked for the pet trade.  Callithrix species have a high invasive potential with allochthonous populations established.  This has led to hybridization of the species and poses a risk for genetic extinction.  All the species face population decline due to loss of habitat.  The three most threatened are C. flaviceps, C. aurita, and C. kuhlii.  Brazil has devised legal instruments to protect and conserve the Callithrix species including Conservation of Endangered Species National Action Plans, a studbook that tracks genealogical relationships in captive C. aurita populations, and the creation of the Center for the Conservation of Saguis-da-serra – a primatology center devoted to developing in situ and ex situ conservation activities.

QUESTIONS

1. What placental and vascular features in marmosets lead to chimerism?
2. What technology has been used to generate transgenic marmosets?
	1. ZNFs
	2. CRISPR/Cas9
	3. TALENs
	4. CRISPR/Cas8
3. Which 3 endangered Callithrix species are most threatened?
4. Which reverse zoonotic disease is fatal to marmosets?
	1. Chikungunya virus
	2. Oropouche virus
	3. Human Herpesvirus 1
	4. Human Herpesvirus 2
	5. Epstein-Barr virus

ANSWERS

1. Marmoset litters usually consist of twins or triplets that share a bidiscoid placenta with fused chorion and vascular anastomoses.
2. b. CRISPR/Cas9 gene knockins have generated transgenic marmosets to model human disease.
3. C. flaviceps, C. aurita, and C. kuhlii
4. c. Human Herpesvirus 1



# **Saravanan et al. Research Relevant Conditions and Pathology in Nonhuman Primates, pp. 139-166**

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

\*NOTE: The majority of this article is presented as various charts, which are challenging to reproduce as a summary. It’s highly recommended to refer to the tables in the published article for review.\*

SUMMARY: Nonhuman primates (NHPs) are used extensively in biomedical research; however they comprise a small proportion of animals used in research overall. NHPs provide good translational models given their close relation to humans, but high cost, low availability, and ethical considerations make NHP research use challenging. Experimental group sizes are often small which can make interpretation of data difficult. It is crucial that veterinary pathologists and clinical veterinarians be able recognize and differentiate between microscopic incidental or background changes and changes induced by experimental disease or treatment. This review article summarizes and presents the common background histologic lesions in the most frequently used NHP species: macaques (rhesus and cynomolgus), baboons, African green monkeys, tamarins, squirrel monkeys, and marmosets. For each species there is a table presenting an overview of morphological background findings by body system. There is also a summary of findings typically associated with immunosuppressive conditions in macaques. The authors provide a brief description of taxonomy, significant spontaneous diseases, and common research uses for each species to supplement the tables.

QUESTIONS

1. Compared to Indian origin rhesus macaques, Chinese rhesus have a/an \_\_\_\_\_ survival rate after infection with SIV.

a. Higher

b. Lower

c. Equal

2. Which of the following infectious causes of diarrhea causes small intestinal villus atrophy in rhesus macaques?

a. Salmonella spp

b. Campylobacter spp

c. Yersinia spp

d. Shigella spp

3. Lymphoid proliferation in the spleen and mesenteric lymph nodes along with lymphoid follicle development in the bone marrow of a rhesus macaque is associated with which infectious agent?

a. Cytomegalovirus

b. Mycobacterium tuberculosis

c. Simian immunodeficiency virus

d. Simian betaretrovirus (SRV)

4. True or False: Lymphoid aggregates may be present in female rhesus vaginas unrelated to inflammation.

5. Which of the following is NOT an age-related histological change in rhesus macaques?

a. Hepatic coagulative necrosis

b. Chronic glomerulonephritis

c. Valvular endocardiosis

d. Subpleural bullae

6. Cynomolgus macaques from which country are preferred for transplantation research due to their limited MHC diversity, which is a result of their descent from a small founder population?

a. India

b. Cambodia

c. Mauritius

d. China

7. Eosinophilic granulocyte infiltrates in epicardial adipose tissue is considered a background finding in which species?

a. Rhesus macaques

b. Cynomolgus macaques

c. Baboons

d. African green monkeys

8. Which herpesvirus causes both intranuclear and intracytoplasmic inclusion bodies in macaque tissues?

a. Macacine alphaherpesvirus 1 (Herpes B virus)

b. Cercopithecine alphaherpesvirus 9 (Simian varicella virus)

c. Macacine betaherpesvirus (Cytomegalovirus)

d. Human alphaherpesvirus 1 (Herpes simplex virus)

9. Infection with SIV in macaques may cause which of the following pathological changes?

a. Pulmonary artery thrombosis

b. Lymph node follicular hyperplasia

c. Maculopapular rash

d. All of the above

e. None of the above

10. Lymphoplasmacytic myocarditis is associated with what infectious agent in baboons housed outdoors in the Southeast United States?

a. Encephalomyocarditis virus

b. Trypanosoma cruzii

c. Simian T-lymphotropic virus type 1

d. Malaria faciparum

11. True or False : Baboons are considered a single species with 5 subspecies, and there are significant genetic differences between the subspecies.

12. Vero cells, a commonly used cell line in biomedical research, are derived from kidney epithelial cells of which species?

a. Macaca mulatta

b. Homo sapiens

c. Papio hamadryas Anubis

d. Chlorocebus aethiops

13. Vitamin A toxicity in African green monkeys leads to \_\_\_\_\_\_\_.

a. Hypertrophy and hyperplasia of hepatic stellate cells

b. Hemolytic anemia

c. Renal papillary edema and fiber deposition

d. Vesicular and ulcerative epithelia lesions

14. Which of the NHP species listed below has a small body size, short dense fur, phalanges ending in claws, and incisors that are larger than the canine teeth?

a. Saguinus spp

b. Callithrix spp

c. Aotus spp

d. Saimiri spp

15. Skeletal muscle degeneration associated with pyogranulomatous pansteatitis in marmosets is found with what vitamin deficiency?

a. Vitamin A

b. Vitamin E

c. Vitamin C

d. Vitamin K

16. Which NHP species has been an informative model for chronic colitis and colon cancer?

a. Callithrix jacchus

b. Saimiri boliviensis

c. Saguinus Oedipus

d. Macaca fasicularis

17. Which virus causes lymphoproliferative disease in owl monkeys?

a. Simian T-lymphotropic virus

b. Simian immunodeficiency virus

c. Saimiriine alphaherpesvirus 1

d. Saimiriine gammaherpesvirus 2

18. Which virus causes multisystemic necrosis and mucocutaneous ulceration in owl monkeys?

a. Simian T-lymphotropic virus

b. Simian immunodeficiency virus

c. Saimiriine alphaherpesvirus 1

d. Saimiriine gammaherpesvirus 2

19. All of the following changes are common in older owl monkeys EXCEPT:

a. Salivary gland adenocarcinoma

b. Chronic nephropathy

c. Left ventricular hypertrophy

d. Multisystemic eosinophilia

20. True or False: Saimiri scuireus are resistant to infection with malaria and are prone to developing gallstones.

ANSWERS

1. a

2. b

3. d

4. True

5. a

6. c

7. b

8. c

9. d

10. b

11. True

12. d

13. a

14. b

15. b

16. c

17. d

18. c

19. a

20. True

# **Colman et al. Marmosets: Welfare, Ethical Use, and IACUC/Regulatory Considerations, pp. 167-178**

Domain 5: Regulatory responsibilities

Secondary Species: Marmosets/Tamarins (Callitrichidae{

SUMMARY: The use of marmosets in research in on the rise, particularly in the United States. Compared to Old World NHP models there are many attractive features of marmosets as a model system, including their high fecundity, short lifespan, small size, rapid maturation, genetic and physiologic similarity to humans, ease of gene editing and in vitro fertilization efficiency, and the similarities to human social structure. Marmosets have been used in biomedical research for decades in research related to neuroscience, infectious disease, toxicology, drug development, gene therapy, reproduction, biodefense, and transgenics and genome editing. Given the rapid increase in demand for marmoset research models, particularly in neuroscience studies, this article aims to provide a summary of the ethics and regulations associated with keeping marmosets in captivity for scientific use. There is discussion of the history and current uses of marmosets in research, ethical considerations, and marmoset-specific research regulations. The authors then present the details of an international survey sent to marmoset users that was developed to gain insight into the main concerns regulatory/oversight bodies have regarding biomedical research with marmosets.

The authors sent the survey to 40 institutions, and received feedback from 18 facilities housing marmosets for research. The majority of the respondents were from institutions within the United States and Canada, with a couple respondents each from Europe and Asia. Survey results showed that the most common area of research using marmosets was neuroscience, and that overall research with marmosets was increasing or stable at the majority of institutions. One institution indicated that research with marmosets was decreasing. All respondents indicated that marmoset research at their institution required review and approval by either an institution-wide committee or an NHP-specific oversight board. Specific topics of consideration for marmoset use by these review groups included appropriateness of marmosets as a model for the specific research, number of marmosets requested, social housing and cage design, environmental enrichment, diet, sanitization  procedures, specific experimental procedures planned, blood volume limits, use of anesthesia vs conscious restraint for procedures, and anesthesia and analgesia plan associated with planned experiments. The specific research regulations for captive marmoset colonies varies by country and the results of the survey highlight some of these differences. In the United States, NHP housing space is determined by animal weight and the requirements only specify floor space, however in Europe the regulations include considerations for group size and the ability to create a complex environment, while also prioritizing vertical space over floor space for arboreal species. Some of the US-based institutions mentioned that they have specific IACUC exemptions for marmoset floor space requirements in order to preserve family units and/or or allow the use of caging with technically smaller floor space but overall increased cubic/vertical space. Some other concerns raised by IACUC/oversight groups at the respondent institutions included questions about the specific procedures to be performed on the marmosets as part of the planned experiments, justification for single housing, adapting sanitization procedures to allow for maintenance of familiar smells during cage/room cleanings, conscious vs chemical restraint for procedures and balancing the refinement of conscious restraint with the potential stress involved in awake procedures. The survey ended with some open response-style questions about the specific concerns related to marmoset research at each institution and how the IACUC or institutional oversight for marmosets compares to that for other NHPs and other lab animal species. As expected, the answers varied widely among the different institutions and highlights the need for shared expertise and establishment of accepted guidelines to help refine the use of marmosets in research.

QUESTIONS

1. What species of marmoset is most commonly used in biomedical research?
	1. Callithrix geoffroyi
	2. Callithrix jacchus
	3. Callithrix penicillata
	4. Callithrix flaviceps
2. Which of the following nonhuman primates is lissencephalic?
	1. Rhesus macaque
	2. Chimpanzee
	3. Marmosets
	4. Squirrel monkeys
3. Which of the following is an important ethical consideration when designing a marmoset research program?
	1. Options for vertical space because the species is highly arboreal.
	2. Scent marking behavior, and the importance of maintaining familiar scents during routine cleaning/husbandry.
	3. Opportunities for social housing.
	4. Blood collection volume limits
	5. All of the above
	6. None of the above
4. Current transgenic marmoset models include all of the following except:
	1. Parkinson’s disease
	2. Stroke
	3. Severe combined immunodeficiency
	4. Retinal degeneration
5. Age-related increases in resistance to what hormone has been identified in marmosets?
	1. Insulin
	2. Cortisol
	3. TSH
	4. Progesterone
6. T/F: Marmosets have the highest fertility of any primate.

ANSWERS

1. b
2. c
3. e
4. d
5. a
6. True

**Ross et al. Marmoset Metabolism, Nutrition, and Obesity, pp. 179-187**

Domain 4: Animal Care

Secondary Species: Marmoset/Tamarins (Callitrichidae)

One-Line Summary: This review article presents what is currently known about nutrition, metabolic dysfunction and obesity in captive marmosets.

SUMMARY: Marmosets are omnivorous animals. In the wild, they consume fruits, gum-based exudates, nectar from over 2 dozen species of plants, insects, and small vertebrates.  In captivity there is no established standardization of dietary components and base diets vary significantly among institutions.

The authors circulated a survey comprising 29 questions to 39 institutions known to house common marmosets.  The survey sought information on current colony census, weighing practices, current weight data, diets used, and opinions regarding limits for healthy, obese, and underweight determinations. Overall, 18 (46%) institutions sent in responses to the surveys sent.

Findings from the surveys include that colony sizes ranged from 10 to 542 marmosets.  Adult females weighed more than adult males or older males with the actual figures being: adult males 414.7 ± 2.9 g, adult females 427.8 ± 3.9 g, older males 398.0 ± 3.9 g, and older females 423.3 ± 7.9 g.

Metabolic function and obesity in captive marmosets were then investigated by the authors.  They carried out several experiments and found that marmosets fed diets higher in glucose had a significant increase in body and fat mass after 16 weeks, whereas marmosets fed a diet higher in fat displayed no consistent increase in fat mass.

The used a device called a lickometer to collect data concerning feeding choices.  The results from the lickometer trials indicated that the amount of food consumed, food choice (high fat vs low fat), grams per lick, and length or number of meals did not differ for an individual over time. Females displayed shorter lickometer meal lengths and slight decreases in overall solid food caloric intake. Adult animals with a propensity towards obesity ingested more food at a single bout than other animals.

In infants or juvenile marmosets, the development of obesity prior to 1 year of age was also associated with impaired metabolic function. Obese infants were found to have significantly reduced insulin sensitivity by 6 months of age.  Infants that were trending towards obesity by 6 months of age began to develop metabolic dysfunction and by 1 year of age displayed typical physiological markers of metabolic disease. They were shown to take larger volumes per lick of the sipper tubes rather than accessing the licksits more frequently or consuming more calories overall. This behavior was stated to be equivalent to the propensity in obese humans of consuming larger bites of food rather than increasing the prevalence of feeding or increasing caloric intake.

Irritable bowel disease and obesity remained a concern, in the authors’ opinions, for captive colonies, and etiologies for these conditions remained unknown. Investigations to evaluate the development of obesity suggested that marmosets spontaneously develop obesity and there were a number of variables that can predict the risk of becoming obese, including preference for high-fat milk and lickometer sip size in these animals.

QUESTIONS

1.   Why is the marmoset, among other non-human primates, used in research?

2.  Inflammatory Bowel Disease is known by other names in Marmosets.  What are those names?

3.  What are the two digestive diseases or conditions the article emphasizes occurring predominantly in captive marmosets?

4.  What is a Lick counter as described in the article?

ANSWERS

1.  Marmosets are used because of their small size, ease of handling, rapid reproductive maturation, high fecundity, compressed life cycle, social behaviors and communication that closely resemble those observed in humans. Additionally, like other NHPs, their genetic similarity to humans.

2.  Marmoset wasting syndrome, Colitis, IBD, chronic diarrhea, and Chronic Lymphocytic Enteritis (CLE).

3.  Inflammatory Bowel Disease and Obesity

4.  A Columbus Instruments DM-8 lick counter was adapted to count the number of times an animal accessed a standard water bottle containing the liquid base purified diet with xanthum gum as an emulsifier.

# **Sheh. The Gastrointestinal Microbiota of the Common Marmoset (Callithrix jacchus), pp. 188-198**

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

Secondary Species: Marmoset/Tamarins (Callitrichidae)

SUMMARY:Gastrointestinal diseases, such as IBD, are the most common and widespread clinical finding in captive common marmosets. With the possible association of stress and diet on the microbiome as well as the advance in sequencing- based microbiome studies, there is interest to understand the role of microbiome in healthy and diseased marmosets.

This systematic review evaluates publications to summarize the current knowledge of the marmoset microbiome and its association with specific GI diseases. In healthy marmosets, the most abundant phylum reported across multiple institutions are *Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria or Proteobacteria*. There is a large plasticity of marmoset gut microbiome depending on the diet, husbandry, and environment. It was also noted that the microbiome profiles from different sources did not completely converge but maintained distinctive characteristics according to the original source of importation. In diseased marmosets, the microbiota has been evaluated in marmoset models of EAE (MS- like disease), CalHV3 and marmosets with duodenal strictures and diarrhea. Increased levels of *C. perfringens* were observed in marmosets with strictures. Overall, there is a large range of variability in the gut microbiome of captive marmosets, observed in both healthy and diseased.  Additionally, there are other enteric bacteria, such as *Salmonella,*enteropathogenic*E. coli, Campylobacter, Yersinia enterocolitica, Shigella sonnei,*and*Klebsiella pneumonia* that can cause GI disease, associated with disruption of the microbiome. Current reports suggest the importance of diet, importation, source and/or environment, age and co-housing to contribute to the composition of the microbiome. Additionally, sample collection and library preparation (DNA extraction techniques, 16S rRNA primers selection, protocols and bioinformatics) need to be considered as sources of potential variation between studies. Also need to incorporate higher resolution metagenomics sequencing data in addition to 16S rRNA surveys, to provide better resolution of bacterial taxa and functions carried out by these bacteria.

QUESTIONS

1. T or F: Metagenomics data will provide data regarding viral, fungal, and parasitic communities.
2. Which bacteria in the common marmoset has been found to possess unique genes, like ABC transporters, that may contribute to their persistence in the gut and role in marmoset nutrition?
*a. Firmicutes*

*b. Bifidobacteria*

*c. Proteobacteria*

d. all of the above

ANSWERS

1. True
2. b.

**Fitz et al. Clinical Management of Gastrointestinal Disease in the Common Marmoset (*Callithrix jacchus*), pp. 199-217**

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

**Secondary Species:** Marmoset/Tamarins (Callitrichidae)

**SUMMARY:**This article describes the management, diagnosis, and treatment of gastrointestinal disease in marmosets. In order to identify gastrointestinal (GI) diseases, facilities should establish routine colony surveillance methods, including daily health evaluations, inspection of the enclosure, weight and body condition monitoring, and fecal scoring. These practices will help with the early identification of GI abnormalities allowing for timely intervention.

The article describes the different causes of GI disease in marmosets, including:

* **Housing Density and Stress:** Increased density in the room and introduction of new animals
* **Diet:** Inadequate dietary protein and immune reaction to the wheat protein gliadin
* **Bacterial:** Campylobacter jejuni, Campylobacter coli, Clostridium difficile, Escherichia coli, Klebsiella pneumoniae, and Salmonella spp. as well as dysbiosis associated with diet, antibiotic use, stress, and GI motility
* **Viral:** Callitrichid herpesvirus 3 (CHV-3) is associated with lymphoproliferative disease affecting the GI tract as well as other organs
* **Parasitic:** Giardia intestinalis and Cryptosporidium parvum
* **Neoplastic:** Small intestinal adenocarcinoma and GI lymphoma
* **Inflammatory:** Amyloidosis
* **Unknown Etiology:** Marmoset duodenal dilation syndrome is characterized by dilation and obstruction of the proximal duodenum as the result of adhesions. Inflammatory bowel disease is categorized into two entities: chronic lymphocytic enteritis (CLE) and large bowel colitis. CLE is characterized by the infiltration of T-lymphocytes into the lamina propria of the small intestine.

GI disease often manifests as non-specific clinical signs, including weight loss and diarrhea, so additional diagnostic tests are necessary to identify the causative agent. Diagnostic tests include fecal analysis (culture for bacteria, flotation, smear, and antigen for parasites), hematology/chemistry/urinalysis (for dehydration and inflammation), and imaging (for masses and GI-associated metabolic bone disease). Several biomarkers (cobalamin, folate, N-methylhistamine, albumin, MMP9, calprotectin, alpha-1 proteinase inhibitor, fecal fat) are currently being investigated for use, but further investigation is necessary.



Treatment is based on providing supportive care to maintain food and water intake as well as other treatments for specific causes (antibiotics, anti-inflammatory medications) or symptoms (analgesia, gastroprotectants, anti-nausea medications).

**QUESTIONS**

1. What is the most commonly reported neoplasia found in the gastrointestinal tract of marmosets?

a. Lymphoma

b. Small intestinal adenocarcinoma

c. Gastrointestinal stromal tumor

d. Leiomyosarcoma

2. Chronic lymphocytic enteritis is associated with infiltration of what type of cells into the lamina propria?

a. Neoplastic

b. B-cell

c. T-cell

d. Neutrophils

3. Thickened intestines or masses may be palpated in which of the following disease processes?

a. Chronic lymphocytic enteritis

b. Small intestinal adenocarcinoma

c. Marmoset duodenal dilation syndrome

d. GI lymphoma

e. Callitrichid herpesvirus-3

f. All of the above

**ANSWERS**

1. b. Small intestinal adenocarcinoma

2. c. T-cell

3. f. All of the above

# **Goodroe et al. Current Topics in Marmoset Anesthesia and Analgesia, pp. 218-229**

Domain 2: Management of Pain and Distress

Secondary Species: Marmoset/Tamarins (Callitrichidae)

SUMMARY: Marmoset use in biomedical research has increased; small size and unique anatomy and physiology make anesthetic and analgesic practices challenging in this species.  The authors compiled a review of current anesthetic and analgesic regimens for marmosets; published dose regimens for anesthetic and analgesic drugs and perianesthetic options are included.  While this review provides a very useful reference for veterinary and research personnel, the authors highlight the continued need for evidence-based resources specific to marmosets to further refine anesthetic and analgesic activities in order to accommodate their unique needs and provide adequate anesthesia and analgesia.

QUESTIONS

1. \_\_\_\_\_\_\_\_\_\_\_ is central nervous system depression and relaxation, but patients may be responsive to noxious stimuli.

a. Tranquilization

b. Sedation

c. Anesthesia

d. General anesthesia

2. \_\_\_\_\_\_\_\_\_\_\_\_ is the “loss of sensation to the entire or any part of the body.”

a. Tranquilization

b. Sedation

c. Anesthesia

d. General anesthesia

3. \_\_\_\_\_\_\_\_\_\_\_\_\_ refers to unconsciousness achieved through reversible depression of the central nervous system.

a. Tranquilization

b. Sedation

c. Anesthesia

d. General anesthesia

4. Which of the following IS true about ketamine?

a. It is a phencyclidine derivative

b. It has a wide safety margin

c. The low pH of formulations has led to myotoxicity and elevations in creatine kinase and aspartate transaminase

d. None of the above are true

e. All of the above are true

5. Which of the following IS NOT an α2 adrenergic receptor agonist?

a. Dexmedetomidine

b. Xylazine

c. Ketamine

d. Medetomidine

6. What type of drugs are Diazepam and midazolam?

a. Benzodiazepines

b. Opioids

c. NSAIDs

d. NMDA antagonists

7. Yohimbine and atipamezole are used to reverse the effects of which drug type?

a. Opioids

b. NMDA antagonists

c. α2 adrenergic receptor agonists

d. Benzodiazepines

8. Which of the following IS the tidal volume of marmosets?

a. 1 to 3 ml

b. 2 to 4 ml

c. 5 to 7 ml

d. 7 to 9 ml

9. In general, how long are marmosets fasted prior to induction of general anesthesia?

a. 1-4 hours prior

b. 6-12 hours prior

c. 18-24 hours prior

d. Not generally fasted prior to general anesthesia

10. Opioids interact with which of the following receptors?

a. Mu

b. Kappa

c. Delta

d. All of the above

e. None of the above

11. What type of drug is maropitant?

a. A serotonin receptor antagonist

b. A proton pump inhibitor

c. A neurokinin receptor antagonist

d. A histamine type 2 receptor antagonist

12. What type of drug is famotidine?

a. A serotonin receptor antagonist

b. A proton pump inhibitor

c. A neurokinin receptor antagonist

d. A histamine type 2 receptor antagonist

13. What type of drug is ondansetron?

a. A serotonin receptor antagonist

b. A proton pump inhibitor

c. A neurokinin receptor antagonist

d. A histamine type 2 receptor antagonist

14. What type of drug is omeprazole?

a. A serotonin receptor antagonist

b. A proton pump inhibitor

c. A neurokinin receptor antagonist

d. A histamine type 2 receptor antagonist

ANSWERS

1. b

2. c

3. d

4. e

5. c; NMDA Antagonist

6. a

7. c

8. c

9. b

10. d

11. c

12. d

13. a

14. b

**Schaeffer et al. February 2021. Magnetic Resonance Imaging of Marmoset Monkeys. ILAR Journal, ilaa029,**[**https://doi.org/10.1093/ilar/ilaa029**](https://doi.org/10.1093/ilar/ilaa029)

Domain 3: Research

Secondary Species: Marmoset/Tamarins (Callitrichidae)

SUMMARY: This article reviews the practical aspects of using MRI and fMRI (functional MRI) to study brain architecture in marmosets. The use of MRI in marmosets has increased rapidly in recent years and is expected to continue to grow. Like macaques (the NHP species traditionally used to study electrophysiology), marmosets also have an elaborated frontal cortex analogous to the human brain. However, marmosets are smaller than macaques and do not carry Herpes B virus, which makes them easier to handle for MRI studies. Marmosets also have a lissencephalic cortex, which is well suited for invasive methodologies that can be paired with MRI.

Marmosets can be imaged using either specialized small-animal MRI scanners (“small-bore” systems typically used in rodents) or “large-bore”, clinical-type MRI scanners (typically used for humans). Small-bore scanners are not optimized for the larger marmoset head/brain and may require specialized coils. Two advantages of using large-bore scanners are the short scan times and the ability to image marmosets in an upright position.

Head movement is a major challenge to obtaining high-quality MRI scans. This can be reduced by anesthetizing the animal or using head fixation. Anesthesia has deleterious effects on neural activity and Isoflurane (the drug most commonly used to anesthetize marmosets for MRI) and propofol have been shown to cause dose-dependent reductions in systemic blood oxygen levels, which may affect some MRI signals. Although anesthetized, breathing may still create motion that impairs the quality of the MRI image. For this reason, even anesthetized animals should be head fixed. Head fixing in awake animals requires training and acclimation that can take at least a few weeks. Animals should be acclimated at increasing intervals to body restraint, then mock MRI tube and MRI sounds, and finally head restraint. MRI of awake, head-fixed animals can be utilized with a surgically implanted chamber, or non-invasive 3-D printed helmets.

The marmoset brain atlas has been created and should be utilized to compare results across individual marmosets.

QUESTIONS

1. Which of the following species does NOT have a lissencephalic cortex?
	1. Mus musculus
	2. Callithrix jacchus
	3. Macaca mulatta
	4. Rattus norvegicus
2. Which of the following is a benefit to using large-bore MRI scanners in marmosets?
	1. Ability to use the sphynx position
	2. Animals do not require anesthesia
	3. Animals can sit upright
	4. They were designed to be used with marmosets
3. T/F: Movement caused by breathing does not interfere with the quality of MRI scans.

ANSWERS

1. c. Macaque

2. c. Animals can sit upright

3. False

**Murai et al. The Importance of Complementary Collaboration of Researchers, Veterinarians, and Husbandry Staff in the Successful Training of Marmoset Behavioral Assays, pp. 230-247**

Domain 3: Research; T1. Facilitate or provide research support; T2. Advise and consult with investigators on matters related to their research; T3. Design and conduct research

Secondary Species: Marmoset/Tamarins (Callitrichidae)

QUESTIONS

1.   T/F: Interest in marmosets as research models has seen exponential growth over the last decade.

2.   T/F: The spectrum of human disease traits that present naturally in marmosets makes them ideally suited as translational models for behavioral and cognitive disorders.

3.   T/F: The range of analogous human behaviors that can be assessed in marmosets makes them ideally suited as translational models for behavioral and cognitive disorders.

4.   T/F: Disorders of the central nervous system commonly manifest as a spectrum of behavioral changes that often serve as the diagnosis for these disorders.

5.    The DSM-5 is the standard reference used to identify behavioral criteria that define the diagnosis of neurodevelopmental, neuropsychiatric, and neurodegenerative disorders… what is the DSM-5?

6.   T/F: With neurodevelopmental, neuropsychiatric, and neurodegenerative disorders for which there is no definitive known cause, the diagnosis is often based purely on behavioral criteria.

7.    What significant spectrum disorder is diagnosed in humans based on behavioral criteria that include deficits in social communication and social interaction across multiple contexts as well as restricted, repetitive behaviors, including stereotypies, which are often accompanied by intellectual disabilities such as impaired learning, memory, and attention?

8.  Neuropsychiatric disorders such as depression and anxiety also include alterations in which of the following:

a.  Social behaviors

b.   Reduced motivation

c.  Alterations in sleep and appetite

d.   Impaired cognition

e.   All of the above

9.    Marmosets have been used as research models for a number of human diseases due to which of the following:

a.  Genetic diversity in line with heterogeneous human patient populations

b.  Physiological systems (e.g., immune, metabolic) similar to humans

c.   Behavioral repertoire analogous to humans

d.  Phenotypic changes related to disease manifestation that can be readily assessed in the laboratory

e.  All of the above

10.  Anatomical similarities of the marmoset brain to the human brain are of great importance for translational research into behavioral and cognitive disorders… what key brain region controls highly advanced cognitive functions and makes marmosets excellent at performing cognitive tasks across several domains, including learning, memory, and attention?

11.   T/F: The prefrontal cortex occupies greater than 10% of the cortex in both humans and marmosets.

12.   T/F: The marmoset brain features a relatively smooth topology, with less sulci and gyri than other primate species, including humans.

13.   The relatively smooth topology of the marmoset brain may confer limitations in which of the following (choose all that apply):

a. Functional mapping studies

b.   Studies of the visual system

c.    Cognitive function such as memory retention time in spatial working memory tasks

d.  Inability to perform multiple cognitive tasks within a given day

14.  T/F: Sex differences across cognitive domains that have been reported in human studies have also been reported across cognitive behaviors in marmosets.

15.   T/F: Marmosets have color vision similar to humans and can use nearly identical modalities for cognitive assessments such as performing CANTAB assays on a touchscreen.

16.   T/F: Marmosets manifest individual differences in responses to anxiety provoking and demonstrate sensitivity to motivational variables, which relate to translational components of neuropsychiatric behavioral criteria.

17. Marmosets have a broad and sophisticated social repertoire, including which of the following (choose all that apply):

a.  Pair bonding

b.   Cooperative care of young

c.  Food sharing

d.   Observational social learning including imitation

18.  T/F: Marmoset demonstrate a rich and complex range of vocal communications, including response calling.

19.   T/F: Similar to humans, marmosets are primarily diurnal with minimal nocturnal activity, which positions them as an excellent model for sleep-related disease co-morbidities.

20.   T/F: Many of the naturally occurring behaviors in marmosets are analogous to human behaviors, suggesting they can be used to study the behavioral hallmarks, progression, and correlation with biomarkers in marmoset models of human diseases.

21.   Marmosets have recently emerged as improved model systems of aging and aging related disorders relative to rodent models?

22.  Marmosets have a relatively short lifespan (<16 years) compared with other NHP species, and data converging from several laboratories have led to a general agreement in the field that marmosets are considered “aged” by…?

a.   8 years

b.   9 years

c.    10 years

d.   11 years

e.   12 years

23.  Marmosets have a relatively short lifespan (<16 years) compared with other NHP species, with an age equivalent ratio of marmoset aging to human aging of approximately…?

a.  1:6

b.  1:7

c.   1:8

d.   1:9

e.  1:10

24.  In addition to aging-related behavioral and cognitive changes, marmosets also demonstrate which of the following aging-related changes similar to humans (choose all that apply):

a.  Decreases in lean body mass and frailty-related characteristics

b.   Metabolic dysfunction

c.   Increased insulin resistance

d.   Myocardial fibrosis

e.  Pathologies related to cancer, diabetes, and chronic renal disease

25.   Which of the following make(s)s marmosets an ideal NHP model for studying aging-related traits:

a.  Presentation of aging characteristics in marmosets are highly analogous to aging humans

b.  Marmosets present with beta amyloid plaques that manifest naturally with aging

c.  Beta amyloid plaques are most frequently reported in marmosets after 7 years of age

d.   Marmoset beta amyloid has a high sequence homology to human beta amyloid

e.  All of the above

26.  T/F: The timing of how aging phenotypes present in marmosets makes them more efficient and economical for studying aging-related traits compared to other NHP species.

27.  T/F: Taken together, the spectrum of human disease traits that present naturally in marmosets, and more specifically the range of analogous human behaviors that can be assessed in marmosets, make them ideally suited as translational models of behavioral and cognitive disorders.

28.   T/F: Marmoset behaviors have inherent variability that are readily influenced by their environment.

29.   For successful behavioral training goals with marmosets, research teams should closely collaborate with husbandry and veterinary teams to minimize and control for as many variables as possible.

30.   T/F: Individual housing is a less common practice in laboratory marmosets given their highly social nature, and laboratory marmosets are commonly housed as family groups akin to their natural family group living conditions in the wild.

31.  Depending on the research goals, successful behavioral studies in marmosets have been conducted using group-housed, pair-housed, and individually housed subjects.

32.   Group housing of marmosets may present additional logistical challenges for behavioral studies, but it may be necessary for neurodevelopmental studies or for studying specific social interactions.

33.  Two simple methods to facilitate visual identification of individuals within group or paired marmosets in their cages?

34.   T/F: For behavioral studies where mature marmosets are required, a common practice is pair-housing of male–female pairs with appropriate methods to control reproduction (e.g., vasectomy, pharmaceutical contraceptives) if breeding is not part of the research component.

35.  T/F: With marmosets, same-sex pairs are possible within siblings.

36.  Unfamiliar same-sex pairing has been less successful in marmosets due to incompatibility and fighting, particularly among which sex?

37.  Depending on research goals, factors that influence enrollment criteria for subject selection may include age, sex, housing considerations, medical and/or surgical history, health or disease/pathogen status, pedigree, genetic background, drug history (experimental or veterinary treatment), and behaviorally experienced/trained vs naïve… can you add 2 additional behavior or temperament-related factors?

38.  T/F: Determining the compatibility of marmoset cage-mates an important criterion for behavioral studies because many behavioral training tasks are conducted at the cage side.

39.  According to the authors of this chapter, which of the following is ‘ideal’ for marmoset behavioral studies?

a.   Single-housed animals in adjacent cages enrolled on the same study

b. Pair-housed animals with one enrolled on the study

c.   Pair-housed animals with both enrolled on the same study

d.  Group-housed animals with multiple animals enrolled on the same study

40.  T/F: Use of compatible group- or pair-housed marmosets would avoid, for example, daily interferences to the non-study cage mate from study protocols that require extensive interaction with research staff, introduction to novel devices (e.g., touchscreen), or cage delivered reinforcers (e.g., food rewards).

41.  Researchers should work closely with veterinary and husbandry colleagues to identify optimal subjects for enrolment in behavioral studies, and to identify the best housing compatibility configurations… what are the micro- and macroenvironmental compatibilities that should be considered?

42. T/F: Husbandry staffs are often the first line of defense in recognizing abnormal behaviors during daily routine welfare checks and may provide key insights into performance levels of marmosets during behavioral tests.

43. Which daily observations should be communicated by husbandry staff for marmosets?

a.  Food consumption/appetite

b.  Abnormal urine or feces (e.g., discoloration, diarrhea)

c.   Activity levels

d.  Unusual behaviors

e.   All of the above

44. For marmosets on behavioral studies, which additional considerations should be included with respect to coordinating daily activities:

a.  Timing experimental sessions before or after regularly scheduled feedings

b.  Ensuring behavioral testing is completed before the day’s cage changes/sanitations which may be stress inducing

c.   Presentation of daily enrichment

d.  Scheduling of veterinary care (routine or otherwise) to minimize disruptions to testing

e.  All of the above

45.  Detailed record keeping and documentation of daily \_\_\_\_\_\_\_\_ and \_\_\_\_\_\_\_\_\_ activities are essential to understanding the history of each subject used in behavioral testing.

46.  With behavioral testing in marmosets, understand the history of the subject includes not only breeding pedigrees and invasive procedures, but also other daily interventions such as \_\_\_\_\_\_\_\_\_\_?

47. Why is it important to keep the presentation of enrichment consistent with all study subjects, including for those that are individually housed and may require additional enrichment?

48.  T/F: If 1 subject or cage within a study group receives a certain enrichment type, then all subjects of the study should also receive the identical enrichment.

49. To understand whether behavioral testing performance for a given day may have been influenced either positively or negatively by the enrichment, you should document the presentation of the enrichment (e.g., type, time of day) as well as the \_\_\_\_\_\_\_\_\_\_\_?

50.  T/F: Relocating new marmosets into the colony room where behavioral test subjects are housed should be done in coordination with research, husbandry, and veterinary colleagues.

51.  T/F: Reconfiguring cages within a colony room where behavioral test subjects are housed should be done in coordination with research, husbandry, and veterinary colleagues.

52.  Breeding colony rooms often require changes in room census or configuration- while they may seem innocuous to staff, such changes may negatively impact behavioral testing performance for study animals housed within the breeding colony room… what are two ways to avoid this potentially negative impact?

53.  What low-cost solution did the authors successfully implement to address adverse social interactions when line of sight with new or non-familiar conspecifics appeared to result in stress-related consequences in the housing room and separate rooms were not an option?

54.  A pre-emptive intervention plan for the veterinary care of each animal should be developed in consultation with the attending veterinarian and staff in advance of initiating the research plan…. what are the two necessary veterinary circumstances to be addressed in that pre-emptive intervention plan?

55.  The animals’ welfare is always the highest priority- what are two reasons behavioral testing in an unwell animal is not prudent?

56.  Which of the following are examples of predetermined reasons that may warrant suspension of behavioral assessments in affected subjects:

a. Unexplained weight loss

b.  Wounds (e.g., from cage-mates or otherwise)

c.  Presentation of clinical signs of disease (e.g., dehydration, obesity, diabetes)

d.  Dental issues

e.  All of the above

57.  Establishing predetermined reasons that may warrant suspension of behavioral assessments is especially important if behavioral studies are evaluating pharmacological intervention- 2 reasons why?

58.  Why is it important that research staff provide advance notice to husbandry and veterinary staff before evaluating different food rewards?

59.  T/F: Anesthesia, routine blood collections and disease surveillance (tuberculosis testing), and medications may impact behavior and could alter the course of behavioral testing.

60.  T/F: A strong communication and wellness plan for the study subjects on which researchers, husbandry staff, and veterinary colleagues agree is an essential element of a successful behavioral research program.

61.  Habituation is an essential component of acclimating a subject to a novel situation... why is this important in behavioral study subjects?

62.  Changes in husbandry practices (e.g., cage changes, new diet), research practices (e.g., behavioral training), or in caretaker or research personnel may induce anxiety in marmosets... how might this anxiety manifest?

63.   What is a typical husbandry event for marmosets that often involves some level of informal habituation and is often paired with a favorable food reward?

64.  In behavioral research settings, habituation procedures commonly use what form of positive reinforcement to familiarize subjects with new situations?

65.   Which of the following are novel situations in which food rewards have proven useful for habituation in marmosets:

a.   Acclimation to new staff

b.  Training to enter a nesting/transfer box

c.   Training to enter a chair for a behavioral procedure

d.  Use as a designated reinforcer for motivation to learn or complete a behavioral task

e.  All of the above

66. Duct tape is considered by most to be the universal bonding agent… what is recognized as the universal preferred food reward throughout marmoset laboratories across the world?

a. Bananas

b.  Raisins

c.  Mealworms

d.   Marshmallows

e.   Acacia gum

67.   T/F: Use of marshmallows a positive reinforcer for motivation in behavioral training AND as a reinforcer for potentially stressful husbandry or veterinary situations could become problematic.

68.  Marshmallows are high in calories and sugar content (3 kcal and 0.5 g sugar in a small 1 cmx1 cm piece), and a diet with as little as \_\_ grams of added sugar may result in obese-like phenotypes in marmosets:

a. 3 g

b.  4 g

c.  5 g

d.  6 g

e.   7 g

69.  T/F: It would be ideal to identify low-calorie, low-sugar rewards for positive reinforcement in marmosets.

70.  T/F: It would be beneficial to identify alternative rewards where the contexts of veterinary procedures and behavioral training procedures are separate so that when used with behavioral training, the same reward is not also associated with any previous exposure to a potentially stressful procedure.

71.   If its beneficial to identify alternative rewards for veterinary procedures vs. behavioral training procedures, should considerations be made to identify an alternative type of transfer device for behavior work that is a different context than those used for husbandry and veterinary procedures?

72.  With behavioral training in marmosets, positive reinforcement, often in the form of treats as rewards, is a major component of behavioral training success… what is the key to ensuring motivation?

73.  T/F: When identifying salient rewards for marmosets, it is critical to also minimize sugar intake given the incidence of diarrhea and obesity as well as the potential for diabetes in marmoset colonies.

74.  Why should preferences for different types of reinforcement be considered on the individual level with marmosets?

75.   In table 5, the authors present results from offering a diverse variety of potential rewards to a group of 15 marmosets to identify possible food reward alternatives to marshmallows…  which of the following was the only item besides marshmallows to have a 100% acceptance criterion based on subjects retrieving and consuming the food within 10 seconds of presentation?

a Sponge cake

b.  Peanut butter

c.   Bio-Serv Yogurt Drops

d.   Bio-Serv Monkey Dough

e.  Fruit Loops

76.  In table 5, the authors present results from offering a diverse variety of potential rewards to a group of 15 marmosets to identify possible food reward alternatives to marshmallows…  which of the following had the lowest acceptance criterion, preferred by only 1 of the 15 animals tested?

a.  Sponge cake

b.  Peanut butter

c.   Bio-Serv Yogurt Drops

d.   Bio-Serv Monkey Dough

e.  Fruit Loops

77.  T/F: When the authors evaluated food reward alternatives to marshmallows among their test group, it was not uncommon for marmosets to initially reject a food but on re-exposure meet acceptance criteria.

78. When the authors evaluated food reward alternatives to marshmallows among their test group, it was less infrequent to observe a subject that initially accepted a food but rejected it in subsequent trials, which ultimately resulted as “not preferred” ... what is the significance of this observation?

79. T/F: During behavioral testing, measurement of reward latency in addition to response behaviors and response latencies may help to understand the level of motivation or potential change in preference in rewards over time.

80.  T/F: History of previous behavioral training or types of rewards provided in the rearing environment (especially if different from the research laboratory) may contribute to how an animal responds to a reward, thus results are likely to vary across laboratories.

81.  T/F: The authors recommend conducting a reward preference assessment to establish types of rewards to provide for behavioral training with marmosets.

82.   Conducting a reward preference assessment may help establish initial reinforcers that are suitable alternatives to high-sugar marshmallows… how might that benefit husbandry and veterinary staff?

83.  When food or water restriction is used for motivation in behavioral training protocols, an elevated level of communication is required between husbandry, veterinary, and research staff to ensure and confirm delivery or restriction of food or water each day… what is an equally important reason for the elevated level of communication?

84.  When food or water restriction is used for motivation in behavioral training protocols, what is recommended as a simple way to communicate restrictions are in place?

85.  When food or water restriction is used for motivation in behavioral training protocols, what mechanism is recommended to track if the day’s restriction has been completed or is in progress?

86.  Sign off sheets provide confidence that the subjects received their daily requirements and minimize miscommunication or failure to comply with food restrictions…. what are the two methods typically used for ‘food restriction’?

87.   T/F: Whenever food or water restrictions are used for behavioral research, consultation with a veterinarian or nutritional expert is critical to ensure that balanced dietary needs are met while also providing for the use of rewards without excessive sugar.

88.   Liquid rewards offered during behavioral tests (e.g., touchscreen tasks) could be diluted to reduce total sugar intake (e.g., 50% dilution) provided what two preference issues are evaluated and identified?

89.   With use of liquid rewards in marmoset behavioral research, preferences may change with age and their ability to discriminate between dilutions may become more challenging… how might this be addressed?

90.  When optimizing conditions for behavioral testing, the total number of rewards that lead to satiety needs to be known… why?

91.  Which of the following evaluations may be necessary to determine the total number of rewards that lead to satiety when using food rewards with marmosets used in behavioral research?

a.  Assessing the number of rewards consumed prior to a decrease in performance levels relative to historic within-subject values

b.  Evaluating latency to retrieve/consume rewards relative to response latency

c.   Evaluating the breakpoint in a progressive ratio task to determine the level of motivation the subject is willing to achieve to continue working for reward

d.  All of the above

92.  T/F: It is important to consider and coordinate with husbandry staff the time of day of testing relative to feeding to avoid satiety prior to testing.

93.  T/F: It would be counterproductive to set training criteria based on a number of trials in a testing session wherein that set criteria and associated number of delivered rewards may be beyond the satiety level for the number of rewards consumed within a session.

94.   T/F: Rewards may interfere with regular diet, and pre-emptive discussions with veterinary and husbandry staff may help identify and document any potential concerns and proactively avoid them.

95. To reduce staff-associated stress, staff acclimation with marmosets typically involves daily sessions of visits to each cage for approximately \_\_\_\_ minutes, often associated with presentation of a preferred reward.

a.  5-15

b.  5-20

c.   10-20

d.  10-30

e.  15-30

96. While an arbitrary number of days of visits could be considered the criteria for habituation to new staff, it is optimal to establish a priori criteria for these trainings in lieu of a general “number of days”… why?

97.  With marmosets, what two habituation criteria are suggested to evaluate progress in training sessions with new staff?

98.  What variation did the authors report in the time required to establish acceptable behavioral responses in training sessions with new staff?

a.  1 day to 1 week

b.  1 day to 2 weeks

c.  2 days to 1 week

d. 2 days to 2 weeks

99.   What repetitive stereotypy did the authors observe/discover in one of their marmosets that was stress-induced by new environmental stimuli or new staff were in the housing room?

a. Back-flipping

b.   Chewing

c.  Hiding

d.  Pacing

e.  Vocalizing

100. T/F: If a repetitive behavior in marmosets reduces with subsequent visits and is eventually eliminated, the behavior may be viewed as an effect of stress that can be managed through successful habituation procedures.

101.  Training protocols should be developed for acclimating marmosets to behavioral instruments… which of the following are examples that would be included in a training protocol for marmosets?

a. Placing the device on the cage front (as in the case of operant or touchscreen training)

b.  Acclimating the subject to a chair to maintain a fixed body position

c.  Training to readily move into a nesting or transfer box as an easy way to relocate the animal from the cage to the testing room without full restraint

d.  All of the above

102.  T/F: In all cases, typical training procedures in marmosets include step-wise training with subsequent extensions of time with the device or in the chair or transfer boxes.

103.  Training procedures in marmosets could start as an initial 1-minute period, for example, that doubles with each subsequent session provided no additional negative indicators of increased stress are manifested… what is a very common indicator of increased stress in marmosets that is easy to monitor?

104.  When acclimating marmosets to behavioral instruments, the authors recommend developing a priori criteria to determine if the animal has acclimated in lieu using a set number of arbitrary days… what are two a priori criteria that could be used to characterize successful acclimation with transfer box training?

105.  Employing positive reinforcement to encourage successful habituation is typical when acclimating marmosets to behavioral instruments… what is considered ‘ideal’ regarding the choice of rewards for training versus other procedures versus use a reinforcement for behavioral tests?

106. T/F: Consumption of positive reinforcements paired with each habituation session would be part of success criteria for training components when acclimating marmosets to behavioral instruments.

107.  Table 1 provides published methods detailing the specifics of training steps for 4 transfer and chair procedures with marmosets... which of the four listed procedures exhibited a success rate of 100%?

a. Transportation for cognitive tasks using a transfer box

b. Transportation for auditory test using a transfer box

c.  Chair restraint for cognitive tasks

d.  Chair restraint for oculomotor tasks

e. All of the above

108.  What two primary stressors can be avoided if behavioral assessments are conducted with marmosets while in their home cage?

109.  While conducting behavioral assessments with marmosets while in their home cage may avoid certain stressors, which of the following are valid reasons that might warrant isolation training?

a. To prevent interference from non-study cage mates

b. To minimize extraneous visual or audio cues

c.  To minimize sources of distractions while assessing measures of attention

d.  To avoid conflict when rewards are presented to 1 cage mate only (as the training subject)

e.  All of the above

110.  What is it about marmoset behavior that might require training in isolated, sound attenuated environments to minimize extraneous visual or audio cues?

111.  Which attention assessment test used with marmosets did the authors cite as an example where the speed of processing reaction time -a key measure- can be disrupted by extraneous visual and audio stimuli and may not be successful if extraneous stimuli are not well controlled?

a. 5-choice serial reaction time test

b.  Bungalow test

c. Fear-potentiated startle response

d.  Hand-eye coordination test

112. With pair-housed marmosets, presenting rewards to 1 cage mate during cage front testing could induce conflict and fighting over the reward, which could negatively impact the training... what adjustment could be employed to avoid conflict while minimizing separation stress?

113.  T/F: When cage front testing marmosets, using a temporary barrier to separate housing pairs physically but not necessarily visually during testing may benefit more submissive cage mates.

114.  Most behavioral assays published on marmosets to date have primarily been focused on what type of  tasks?

115.  Much of the behavioral assays published on marmosets to date have featured use of operant-based conditioning and/or touchscreen tasks such as those similar to the clinical version of the CANTAB... what does the acronym CANTAB stand for?

116.  T/F: Successful training of marmosets for testing of complex cognitive tasks relies on the collaborative and integrative procedures among veterinary, husbandry, and research colleagues.

117.  Table 2 provides a detailed overview of published literature demonstrating the stepwise training procedures employed prior to progressing marmosets for testing of complex cognitive tasks… what is the practical value of these published references for those planning behavioral training in marmosets?

118.  Table 2 lists 22 publications with an overview of their stepwise training procedures employed prior to progressing marmosets for testing of complex cognitive tasks… how many of the published references report a success rate below 100%?

a.   0

b.  1

c.  2

d.  3

e.  4

119.  T/F: Table 3 provides a detailed overview of the testing criteria for evaluating behavioral phenotypes across the spectrum of cognitive domains in marmosets, including tasks for assessing learning, memory, and attention… ability to perform these tasks is dependent on the successful criteria of initial training steps described in Table 2.

120.  Table 3 lists 11 publications about visual working memory testing in marmosets… how many of the 11 published references report a success rate below 100% based on their definition(s) of success?

a.   0

b.  1

c.  2

d.  3

e.  4

121.  Table 3 lists 7 publications about spatial working memory testing in marmosets… how many of the 7 published references report a success rate below 100% based on their definition(s) of success?

a.   0

b.  1

c.  2

d.  3

e.  4

122. Table 3 lists 12 publications about cognitive flexibility testing in marmosets… how many of the 12 published references report a success rate below 100% based on their definition(s) of success?

a.   0

b.  1

c.  2

d.  3

e.  4

123.  Table 3 lists 3 publications about executive function testing in marmosets… how many of the 3 published references report a success rate below 100% based on their definition(s) of success?

a.   0

b.  1

c.  2

d.  3

e.  4

124.  Table 3 lists 2 publications about attention testing in marmosets… how many of the 2 published references report a success rate below 100% based on their definition(s) of success?

a.   0

b.  1

c.  2

d.  3

e.  4

125. Table 2 and Table 3 present details for published stepwise training procedures and testing criteria for complex cognitive tasks… what is significant about the information presented in Table 4?

126.  Table 4 lists examples of training procedures for non-cognitive behavioral task testing in marmosets… which of the following behaviors is/are referenced in Table 4?

a.   Auditory response

b. Gait

c.  Grip strength

d.  Oculomotor

e.  All of the above

127. T/F: While not all animal models have behaviors analogous to human conditions, marmosets have a rich and diverse repertoire of social, emotional, appetitive, and complex cognitive behaviors that are highly translational.

128. This article provided insight on the critical steps for ensuring successful training of marmoset behavioral tasks, including which of the following:

a.  Procedures for habituation to new research staff

b. Acclimation to transfer cages

c.   Acclimation to testing equipment and reinforcements

d.  All of the above

129. T/F: While much of the information provided in this article covered peer-reviewed references and experiences specific to marmosets, many of the methods and suggestions described may be generalizable to other New World NHP species, such as squirrel monkeys and titi monkeys.

130. T/F: Regardless of particular behavioral testing goals behavioral research exclusive to marmosets, the importance of integrating veterinary and husbandry colleagues as part of the research plan will better enable the success of the project.

ANSWERS

1. True

2.  True

3.  True

4.  True

5.  The Diagnostic and Statistical Manual of Mental Disorders, 5th ed

6.  True

7.  Autism

8.  e.  All of the above

9.   e.  All of the above

10. Prefrontal Cortex

11. True

12.  True

13. c.  Cognitive function such as memory retention time in spatial working memory tasks

d.  Inability to perform multiple cognitive tasks within a given day

14. True

15. True

16. True

17. a. Pair bonding

b. Cooperative care of young

c. Food Sharing

d. Observational Social Learning Including Imitation

18.  True

19. True

20. True

21. True

22. a.  8 years

23. c.  1:8

24. a.  Decreases in lean body mass and frailty-related characteristics

b.  Metabolic dysfunction

c.  Increased insulin resistance

d.  Myocardial fibrosis

e.  Pathologies related to cancer, diabetes, and chronic renal disease

25.  d. All of the above

26.  True- other NHP species could require aging studies to go well beyond the age of 20 years

27.  True

28. True

29. True

30. True

31. True

32. True

33. 1) Non-toxic, semi-permanent hair dye in a variety of colors applied to ear tufts

 2) Colored tags on collars

34. True

35.  True

36.  Females

37. - Aggressive nature

- Anxiety level

38. True

39. c. Pair-housed animals with both enrolled on the same study

40.  True

41. - Within the cage as cage mates (microenvironment)

- Within the colony room as social conspecifics (macroenvironment)

42. True- husbandry staffs are typically the first point of contact of the marmosets each day, and given their knowledge and familiarity with individuals, they often have instincts that can identify atypical behaviors

43. e. All of the above

44.  e. All of the above

45. Husbandry, veterinary

46. Enrichment

47. Consistent enrichment presentation minimizes additional variation across subjects

48.  True

49. Response (e.g., avoidance or favorable reaction)

50.  True - relocating new marmosets into the colony room can cause stress and alter behavior

51. True - reconfiguring cages within the room where unfamiliar marmosets become adjacent to each other can cause stress and alter behavior

52. Either don’t make changes in colony rooms wherein behavior study animals are housed, OR house research subjects in their own study-designated housing room outside of the breeding colony

53.  Visual Barriers (e.g., curtains)

54.  - Routine veterinary care

 - A communication plan if suspension of testing is needed due to unscheduled veterinary intervention

55. - Continuing with use of unwell subjects may yield spurious data that complicates reproducibility

 - Continuing with use of unwell subjects could result in worsened health outcomes

56.  e. All of the above

57.  – The experimental intervention could cause adverse reactions and need to be stopped

- Necessary veterinary treatments could interfere with the experimental intervention

58. If health concerns arise, there is a potential known cause that can be investigated

59. True

60.  True

61. To minimize anxiety

62.  Behavioral reactivity, with transient changes in feeding or activity

63.  Acclimation to enter transfer boxes for cage changes, vet checks, treatments, etc.

64. Food rewards

65. e. All of the above

66. d. Marshmallows

67. True

68. d. 6 g

69. True - see Tables 2 and 5 in the article

70. True

71. Yes - the authors designed and use a transfer box with different textures and cues relative to the typical one used for transferring subjects to potentially stressful procedures

72. Preference

73.  True

74.  Marmosets are phenotypically diverse and may demonstrate individualized preferences

75. c. Bio-Serv Yogurt Drops

76. b. Peanut butter

77. True

78.  Initial acceptance that waned suggests the potential of preferences changing over time, as may be the case with aging

79. True

80. True

81. True

82. If only identified alternatives are used with behavioral training, husbandry and veterinary staff could continue using marshmallows for rewards associated with their procedures, i.e., different food rewards for the different contexts

83. To minimize any errors that could result in welfare issues

84. Special visual signage on the cage cards

85. A signoff sheet

86.  - Reducing total calories

- Reducing sweet fruits or vegetables where sweet rewards are used as reinforcers

87.  True

88. – Identification of liquid reward itself (e.g., apple juice vs grape juice vs banana milkshake)

- Identification of the level of dilution for which the reward still remains salient

89.  Perform preference tests throughout animals’ aging to ensure continued motivation

90.  Knowing the total number of rewards leading to satiety is required to delineate poor performance from lack of interest and motivation

91. d. All of the above

92. True

93. True

94. True

95. e. 10-30

96. Individual Marmosets have different levels of behavioral responses to novel stimuli, so a ‘one size fits all’ number of days may not be appropriate for all subjects

97.  - Evaluate reduction in latency of the marmoset to approach the cage front with each subsequent sessions

- Confirm when subjects readily take and consume rewards from the new staff

98. d. 2 days to 2 weeks

99.  a. Back-flipping

100. True

101.  d. All of the above

102. True

103. Increased calls

104.  - The animal entering the transfer box within 1 minute on presentation

        - Vocalizations (often stress induced) subsequently reduce with each session

105.  rewards used for training should be different from rewards used for other procedures or as reinforcement for behavioral tests

106.  True

107.  e. All of the above

108.  - Stress due to transfer

- Stress due to isolation

109.  e. All of the above

110. Marmosets are highly vocal animals and communicate with high-pitched calls (e.g., trill, phee, twitter) when separated from conspecifics

111. a. 5-choice serial reaction time test

112.  Separate housing pairs physically (e.g., a temporary barrier) but not necessarily visually for the test duration... may benefit more submissive cage mates

113. True

114. Cognitive Tasks

115. Cambridge Neuropsychological Test Automated Battery

116.  True

117. Published references provide guidance for establishing training protocols

118. c. 2

119.  True

120.  d. 3

121.  e. 4

122.  e. 4

123.  c. 2

124.  b. 1

125.  Table 4 provides a sample of several non-cognitive behavioral assays in marmosets in which training protocols have been published

126. e. All of the above

127. True

128. d. All of the above

129. True

130. True

# **Homman-Ludiye and Bourne. 2020. The Marmoset: The Next Frontier in Understanding the Development of the Human Brain. ILAR Journal 61(2-3):248-259**

# **Kaas. Comparative Functional Anatomy of Marmoset Brains, pp. 260-273**

Domain 6: Education; Task 2: Maintain current knowledge and continued competence in laboratory animal medicine

Secondary Species: Marmoset/Tamarins (Callitrichidae)

SUMMARY

Introduction: An outline of the role of marmosets in research, and their recent increase in popularity is presented.  This is attributed to being highly vocal and social.  They are also viable for optogenetic studies.  There is also a very generic outline to the relative cortical structures in primates, although more extensive review of marmoset neuroanatomy is referred to another article.

Defining Architectonic Areas and Nuclei: A thorough history of identification of nuclei, cortical and subcortical structures is made, with minor notation to improved methodology from the simple interpretation of Nissl staining to more complex histochemical and immunostaining procedures that allow for functional mapping of connections, including the middle temporal visual area.

The Visual System - Subcortical Structures: The visual system is the most extensively studied system in primates. Functional differences are outlined in the delineation of the lateral geniculate nucleus between taxa and three functional classes, with focus on the neural layers in regard to ipsilateral or contralateral input.

Visual Cortex - Primary and Secondary Areas (V1 and V2) and Areas of the Third Tier: The primary visual cortex in the marmoset comprises approximately 20% of the surface of the neocortex.  V1 occupies a larger portion of the brain in marmosets than in other primates.  V1 also has well-differentiated sublayers with complex interhemispheric connections that extend further than macaques, but not as far as nocturnal primates.  V2 is common to almost all mammals and contains a complete representation of the contralateral visual hemifield, including the zero-horizontal meridian.

Visual Cortex - The Dorsal and Ventral Streams: The middle temporal visual area contains systematic maps of stimulus orientation and direction of motion responses.  Humans and macaques have face-sensitive patches in the lower temporal lobe for social signaling, while marmosets are located in the caudoventral temporal cortex and as such have similar social visual perception to humans for study.

The Auditory System: The auditory processing of almost all mammals is conserved.  The auditory thalamus of the marmoset has been fully described, and spreads to multiple thalamic nuclei.

Auditory Cortex: Marmosets were key components for the current theories of auditory complex organization. The auditory cortex is divided into 3 primary areas, and there are overlapping auditory areas with visual areas in the frontal lobe.

The Somatosensory System: Marmosets have a unique somatosensory system to account for having claws on all fingers and toes except the great toe (unlike other primates).  Marmosets have hands that are more like paws with reduced somatosensory detection compared to other primates, but they still have conserved anthropoid primate specialized receptors for increased tactile input.  The somatosensory cortex is well-defined and largely similar to other primates.

Sensorimotor Cortex: There is currently experimental evidence that the frontal motor areas in marmosets is similar to other primates.  The posterior parietal cortex is less studied than in other primates, though is likely to function similar to the lateral intraparietal area of other primates.

Other Cortical Areas: A thorough guide of atlases and reviews regarding each anatomical location is outlined.  The concept of “corticalization of function” is used to describe the reduced number of neurons in marmosets when compared to other primates.

QUESTIONS

1. T/F: The visual system is the most extensively studied system in primates.

2. The primary visual cortex in marmosets accounts for approximately \_\_\_\_\_\_\_ of the surface of the neocortex.

a. 10%

b. 20%

c. 30%

d. 40%

3. T/F: Marmosets have a unique somatosensory system to account for having claws on all fingers and toes, except the great toe.

ANSWERS

1. True

2. b

3. True

# **[Schaeffer](https://academic.oup.com/ilarjournal/search-results?f_Authors=David+J+Schaeffer) et al. 2020.** [**Magnetic Resonance Imaging of Marmoset Monkeys**](https://academic.oup.com/ilarjournal/article/61/2-3/274/6149923)**, pp. 274-285**

# **Park and Sasaki. Assisted Reproductive Techniques and Genetic Manipulation in the Common Marmoset, pp. 286-303**

Domain 3: Research

Secondary Species: Marmoset/Tamarins (Callitrichidae)

SUMMARY

Introduction

Unique features making marmosets highly suitable for reproductive engineering studies:

* Small body size of adults (350-450g; easier handling and smaller quantity of test materials)
* Similar social structures to humans (breeding pairs with cooperative rearing)
* Reproductive features – rapid sexual maturation (1.5-2y), larger litter size (2-4 offspring), and short gestation intervals (allows for 2 litters/yr)
* High cognitive function (similar to other NHPs)

Aim: To describe progress of assisted reproductive techniques (ART) and genetic engineering technologies in NHPs, including marmosets

Assisted Reproductive Techniques (summarized in Tables 1 and 2)

*Cycle Synchronization and Ovarian Stimulation* – Marmoset progesterone levels show a mean ovarian cycle length of 28d (~ 8-10d follicular phase:18-20d luteal phase) that can be controlled by administration of cloprostenol (prostaglandin f-2α analog) to induce luteolysis within 24 hours to reset the ovarian cycle and induce ovulation with injection of human chorionic gonadotropin (hCG). Further optimization of the ovarian stimulation protocol is needed to reduce numbers of postmature oocytes (in vivo matured oocytes; IVO) and generate high numbers of competent metaphase II (MII) stage oocytes.  In general, FSH priming combined with timed hCG treatment successfully increases preovulatory follicles and the ability to collect large numbers of viable oocytes in marmosets.

*In Vitro Maturation (IVM)* – Marmoset ovaries possess abundant small and medium-sized antral follicles with oocytes capable of undergoing maturation in vitro under proper conditions such as basal media with protein additives. Negative effects have been shown of both epidermal growth factor (EGF) and high doses of estradiol supplementation on IVM. In current IVM protocols, reliable rates of oocyte maturation are achieved after 26-30 hours incubation.

*In Vitro Fertilization (IVF)* – Successful IVF occurs with co-incubation of mature oocytes and spermatozoa for up to 30hr. The sperm collection method of choice in marmosets is penile vibratory stimulation (PVS) for increased yield of more motile spermatozoa than electro-ejaculation. Spermatozoa are washed and selected using swim-up or density gradient centrifugation.  In vitro capacitation can be achieved by removal of seminal plasma and incubation with media containing protein sources and various ions. After co-culture, zygotes are cleaned to remove adhered sperm and cumulus cells and placed in fresh culture medium.

*In Vitro Culture (IVC)* – Presumptive zygotes are cultured in vitro utilizing various media types and newer “sequential” media systems until the blastocyst stage (up to 2 weeks).  A wide variety of approaches to culture marmoset embryos are available, and it is important to optimize conditions and establish standards to allow comparison of research outcomes across studies.

*Embryo Transfer (ET)* –  Surgical embryo transfer techniques have been successful with fresh or frozen-thawed embryo delivery after laparotomy. For animal welfare, non-surgical transfer of embryos to the uterine fundus is possible by a skilled operator with gentle guidance of a blunt cannula and stylet through the cervical canal into uterine lumen. The non-surgical technique is complicated by the location of the fornix near the external os of the cervix in marmosets, passage through viscous cervical mucus secretions, and damage to uterine endometrium from cannula manipulation. Successful pregnancies and live births have been achieved following transfer of in vitro-produced marmoset embryos to recipient animals with higher success rates when transferring early cleavage stage embryos (prior to morula or blastocyst stage) to asynchronous recipients (cycle/ovulatory stimulation of the embryo donor at least 2 days prior to the embryo recipient), and utilizing a transfer volume of 1µL or less.

*Intracytoplasmic Sperm Injection (ICSI)* – ICSI has been an effective fertilization method for marmosets, and could be utilized to reproduce high-value founders and the production of transgenic animals.

Genetic Manipulation Techniques in Mammals (summarized in Table3)

2 categories:

* Transgenic – insertion of exogenous gene into host genome
* Gene Targeting – introduction of genetic modifications into specific regions of a target gene

*Pronuclei DNA Microinjection* – DNA is injected and randomly integrated into host genome during DNA replication and maintained during embryo development resulting in neonates with foreign DNA.

* Limitations: poor efficiency (~1%) and requires large numbers of oocytes (limited in NHPs)

*Retroviral Vectors* – Vesicular stomatitis virus (VSV-G) or lentivirus vector (SIV or CMV version) have been utilized to successfully introduce green florescent protein (GFP) gene into various NHP species, including rhesus m, cynomolgus m, and marmosets.

*ICSI-mediated Transgenesis* – Spermatozoa incubated with foreign DNA followed by injection into oocytes allows introduction of large transgenes using bacterial or yeast artificial chromosomes with high efficiency; no live NHP offspring, but stillborn animals showed GFP epifluorescence.

*Pluripotent Stem Cells* – Direct injection into host embryos produces “naïve” chimeric competent pluripotent stem cells. However, chimeric competency has not been proven in NHPs, as primitive endodermal cells of blastocysts prevent incorporation of injected cells into host embryos.

* Limitations: unproven production of chimeric NHP, and considered unpractical to produce modified NHP colonies due to prolonged time to next generation

*Germline Stem Cells* – Spermatogonial stem cells (SSGs) have the ability for self-renewal and give rise to spermatocytes, spermatids, and spermatozoa. While they exist at extremely low levels, they can be isolated from testes (adult and neonate) to expand into long-term cultures and transplanted into host testis to produce viable offspring (currently unproven in NHPs).

*Somatic Cell Nuclear Transfer* – Generates genetically identical animals via culture of donor somatic cells and oocytes, transfer of donor cells into enucleated oocytes, artificial activation of reconstructed embryo, and transfer of cloned embryo into recipient.  SCNT can also produce transgenic animals or target gene knock-out/-in animals when using genetically manipulated cells as donor cells.  Various attempts in NHPs has produced no successful cloned animals thus far.

* Limitation: low success for generation of live offspring (2-3%)

*Gene Editing* – While CRISPR/Cas9 system is the most popular method of gene editing, the resultant mosaic embryos with mutant and wild-type blastomeres limits the use in NHPs due to the length of time required (2-5yrs) for obtaining second-generation offspring with objective phenotypes. ZFNs and TALENs do not produce mosaic alterations, and are suitable for gene targeting of NHPs. Base editing techniques have also produced successful disease models in cynomolgus m.

CRISPR = clustered regularly interspaced short palindromic repeat) associated Cas9 system

ZFNs = Zinc finger nucleases

TALENs = Transcription activator-like effector nucleases

Future Perspectives:Cryopreserved sperm and vitrified embryos have been successfully used for producing marmoset offspring, and have important implications in conserving and enabling the exchange of valuable marmoset resources worldwide. Further exploration is needed to development of embryo biopsy techniques for preimplantation genetic screening, as well as SCNT or nuclear transfer techniques to produce founder generation of phenotypic models with faster potential for production of offspring and colony development.

QUESTIONS

1. Published FSH dosages utilized in marmoset studies follows what characteristics:
	1. Comparable to rhesus macaque dosages; double human dosages
	2. Double rhesus macaque dosages; comparable to human dosages
	3. Comparable to both rhesus macaque and human dosages
	4. Up to 12.5 times rhesus macaque dosages
2. The addition of which substance typically utilized in rhesus research protocols for the incubation of spermatozoa resulted in notable hyperactivity followed by rapid loss of motility when adapted to early marmoset IVF studies?
	1. Caffeine
	2. Dibutyryl cyclic AMP (dbcAMP)
	3. A and B
	4. None of the above
3. True/False – When spermatogonial/germline stem cells are cultured under embryonic stem cell culture conditions, they can be converted from unipotent cells only involved in spermatogenesis to pluripotent cells for use to produce chimeric animals.
4. Which animal species was used to produce the first offspring obtained by somatic cell nuclear transfer?
	1. Mice
	2. Rats
	3. Sheep
	4. Nonhuman primate

ANSWERS

1. a. (note – high dosages of rFSH were required for ovarian hyperstimulation in marmosets at 12.5 times those used in rhesus monkeys)
2. c.
3. True
4. c.