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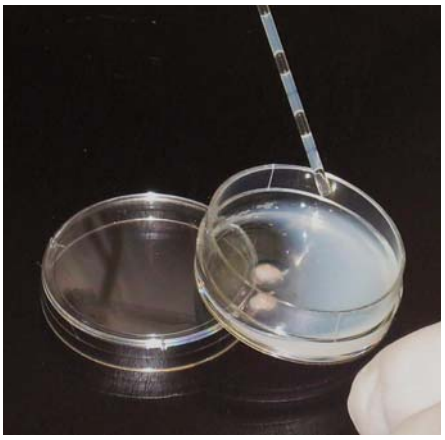
WEILL CORNELL MEDICAL COLLEGE EDITION

In Focus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology



Cryoprotectant being loaded into a straw for sperm freezing.

INSIDE:
USDA Moves to Enforce Regulations
New Staff Intro
Lab Animal Technician Week

Cryobanking of Genetically Engineered Mice

The mouse continues to be the "premier" animal model used at Weill Cornell. WCMC's mouse population has exploded over the past decade as genetically engineered mouse (GEM) strains are extraordinarily valuable animal models. To accommodate this growth, existing vivaria have been modified and expanded, and new facilities planned.

Cryobanking is an integral tool for managing GEM strains as it allows animal housing space to be freed up when strains not in use are cryopreserved. More importantly, cryobanking also serves to protect unique and often irreplaceable GEM strains from loss due to disease or environmental catastrophes. Freezing of mouse sperm has largely replaced embryo cryopreservation, except for complex strains with multiple gene deletions and insertions, as sperm freezing is a less complex procedure to perform. Moreover, recent improved methodology greatly increased the efficiency of live animal recovery from frozen sperm by IVF in many inbred mouse strains[1]. Some of these modifications included the use of improved cryoprotectant and IVF media, a refinement of cooling and warming rates during freezing and thawing, and the storage of frozen samples in straws.

The Mouse Genetics Core Facility (MGCF) in conjunction with the Jackson Laboratory (Jax) recently undertook a major sperm cryopreservation project that involved freezing sperm from more than 100 mouse strains from 15 laboratories. Jax provided a database to track strain information including line name, genetic background, birthdates of sperm donors, coat color, and mouse genotype. The MGCF staff organized and tested the collection of male donors prior to sperm freezing. Jax's cryopreservation team completed the freezing procedure on >200 male mice in 3 days. Frozen sperm samples are stored in two separate locations to protect against loss.

This on-site, large-scale freezing program was a big success as indicated by the high

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Pain and Distress in Lab Animals: Establishing Humane Endpoints

Zoologist William Russell and microbiologist Rex Burch studied the ethics of laboratory research and "the development and progress of humane techniques" in the laboratory setting. The British duo revolutionized the concept of the humane use of animals in research by publishing their 1959 book, *The Principles of Humane Experimental Technique*, which introduced the concepts of The Three R's: Replacement, Reduction and Refinement.

Replacement can be considered as either absolute or relative. The former refers to the replacement of an animal model with a non-sentient model such as plants or micro-organisms. The latter refers to the use of animal-derived cells in vitro, rather than the use of a living animal. Some also consider replacement to include using an animal lower on the phylogenetic scale. The second principle, *Reduction*, refers to minimizing the numbers of animals used in a study. *Refinement,* involves altering experimental techniques to reduce the incidence or severity of pain and/or distress experienced by laboratory animals.

As users of animals in research, it is imperative that we recognize that animals, like humans, feel pain. The International Association for the Study of Pain defines pain *as,* "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Pain can be categorized as physiologic or pathologic; acute or chronic; and, visceral, neurogenic or somatic. Physiologic pain has a protective function and serves as an "early warning sign" to prevent or limit the possibility of further injury by limiting movement or activity. This type of pain may be protective and can often be managed successfully with analgesics. However, excessive or untreated physiologic pain that is associated with tissue damage and inflammation can develop into pathologic pain resulting in detrimental physiological and psychological effects.

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Odessa Giardino, MS, CVT, RLATG
EQA Administrator

Edgar McNab, MS, MT(ASCP)
LCP Manager

Research Animal Surgical Facility is one of the S-3 Facility highlights.

New Staff

Odessa Giardino has joined RARC as head of the Education and Quality Assurance section, filling the vacancy left by Anita Piccolie's retirement. Odessa has extensive experience in the development and design of laboratory animal training programs and regulatory compliance. Most recently she developed a global laboratory animal science training program for Wyeth pharmaceuticals and was Head, Regulatory Compliance and Training at Pfizer.

She will oversee the daily operations of the Education and Quality Assurance section at both MSKCC and WCMC and will be focused on expanding the RARC training curriculum to include e-Learning modules. She can be reached at (212) 746-1077 or email at giardino@mskcc.org or odg2001@med.cornell.edu.

Edgar Mc Nab has joined the Laboratory of Comparative Pathology (LCP) as Laboratory Manager. Edgar brings a wealth of anatomic and clinical pathology laboratory experience having worked on three continents and six countries during his career. Most recently, in association with the CDC and the Clinton Foundation, he assisted in developing a national external quality assurance program for all Ethiopia's 106 diagnostic laboratories.

Edgar began his career as a medical technologist and subsequently has served in a variety of managerial, administrative, and consulting positions. He will oversee the daily operations of both the clinical and anatomic pathology sections of the LCP which supports both MSKCC and WCMC and will oversee the laboratory's continued expansion. He can be reached at (646) 888-2421 or email at mcnabe@mskcc.org or edm2012@med.cornell.edu.

S Building Vivarium to Reopen

The vivarium located on the 3rd floor of the S building is scheduled to reopen in January '11. The vivarium, which has been closed ~ 2 years, has been expanded and completely renovated. The facility will contain the Research Animal Surgical Facility (RASF), holding rooms for both large and small animals, procedure laboratories, and a large animal necropsy. The RASF contains 2 operating rooms, a pharmacy, and a recovery suite. The facility supports large animal model development as well as surgical training courses using large animals.

Laboratory Animal Technician Week

January 30th to February 5th, is International Laboratory Animal Technician Week. This week acknowledges the dedication and efforts of the various types of technicians that support *in vivo* research with animals. These technicians have a variety of roles including sanitizing cages in cage wash, changing cages and feeding and watering animals, providing enrichment, assisting with technical and surgical procedures, as well as treating sick animals in all WCMC's facilities. This year's theme is "Respect". Respect is crucial to our mission. It is important to show respect to the various technicians that support our research programs. Please take a moment during this important week to thank the RARC and laboratory technicians who care for your animals and support your research.

USDA Announces "Age of Enforcement"

The United States Department of Agriculture (USDA) has administered the Animal Welfare Act (AWA) Regulations for more than a quarter of a century. The AWA Regulations require that any facility that houses covered species for use in research be registered with the USDA and requires that all registrants provide animal care that meets or exceeds the USDA's standards for veterinary care and animal husbandry. These standards include housing, feeding, handling, ventilation, and shelter. Covered species include "any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm-blooded animal, which is being used, or is intended for use for research, teaching, experimentation, or exhibition purposes". The regulations **exclude** birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research; and farm animals or poultry used or intended for use as food or fiber or intended for use for improving animal nutrition, breeding,

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Pain and Distress in Lab Animals, cont. from pg. 1

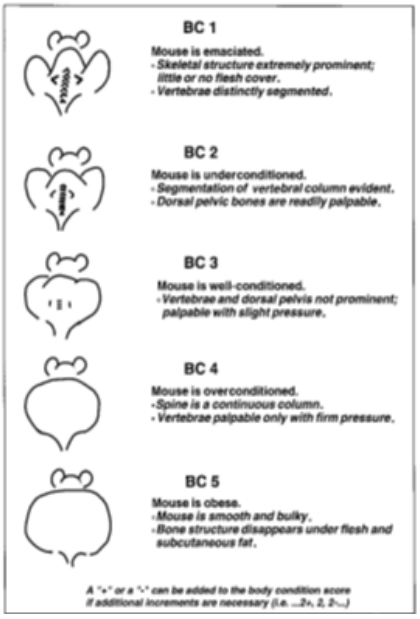
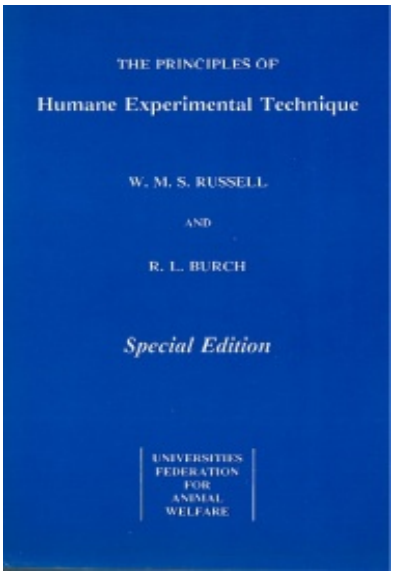


Fig 1. Body scoring system from reference 4.



The classic 1959 publication which introduced the concept of the 3R's.

TABLE I: NOCIFENSIVE BEHAVIOR
Reflexes e.g. Withdrawal reflex
Vocalization
Guarding
Escape
Aggression
Shaking
Rubbing
Scratching
Biting affected area
Paw licking
Looking at affected area

Animals, like infants, are unable to express their pain verbally. This fact makes it more difficult to assess pain in the two groups which led to the now outdated belief that neither animal nor infant are capable of perceiving pain. Early last century, neonatal humans were commonly denied pain medication as it was widely believed that the neonatal neurological system was not mature enough to sense pain.

Animals display pain-like or "nocifensive" behavior (outlined in Table 1). Evidence suggests that nocifensive behavior in animals and humans are generated at very similar thresholds and the magnitude of the response is proportional to the intensity of the stimulus. Therefore, procedures that cause pain or distress in humans should be considered to cause pain and distress in animals until proven otherwise.

Animals display the same motor behaviors shown by humans in response to painful stimuli such as reflexes (e.g. tail flick, withdrawal, and jaw-opening) and vocalization (See Table I). It is important to note that the lack of behavioral response to painful stimuli does not prove lack of pain, rather it may simply be a result of inhibition of motor response or in the case of many laboratory animals an *instinctive stoicism* displayed by many prey animals.

Fear, pain and anxiety can all lead to a state of distress. Stress is a normal biological event which involves adaptive behavior or a physiological process to maintain homeostasis in response to a stressor. When stress becomes excessive or prolonged, the animal, unable to adapt, may become distressed. Distress is usually associated with a change in motility or locomotion, and can result in stereotypic (repetitive) behaviors such as pacing, self-biting and excessive grooming. Animals may experience pain due to a disease, experimental procedure, toxicity etc. Pain alters the animal's physiological and psychological state leading to aberrations in metabolism and immunity. These changes impact not only the animal's welfare, but confound research results.

The "obvious" solution of administering analgesics to animals in pain may be a cause for concern as analgesics, sedatives or anesthetics may interfere with research as they have concurrent physiologic effects. On the other hand, in the event of unrelieved pain/distress, the study may need to be ended early prior to the acquisition of useful data. Study objectives must be balanced against animal welfare considerations. In most research this is achieved by establishing humane endpoints, clearly described in the animal use protocol submitted to the IACUC, prior to commencing the study.

The *Organization for Economic Co-operation and Development* states that "A humane endpoint can be defined as the earliest indicator in an animal experiment of severe pain, severe distress, suffering, or impending death." Humane endpoints should take into account the *cumulative effect* of all experimental effects on the animal.

The challenge is to find the balance between ending an experiment *too early*, thereby obtaining irrelevant research results and wasting the life of an animal, or ending an experiment *too late* causing the animal undue distress and compromising their welfare.

The following general scenarios. viewed as appropriate humane endpoints, can be considered:

1) The animal is so physiologically deranged that its use will no longer generate scientifically useful data (the physiological derangement may or may not be directly related to the variable being studied);
2) The animal is distressed to the point that it will no longer provide scientifically useful data;
3) Suffering to an unanticipated degree has occurred, outweighing the potential benefits of the research;
4) Suffering is so extreme that it is simply unethical to subject an animal to that kind of treatment; and, Research will yield results that "justify" animal suffering, but it is unnecessary to reach that level of suffering to obtain useful data. In these cases earlier events can predict the scientific endpoint.

Humane endpoints also benefit research. Establishing a specified time for euthanasia allows timely collection of needed tissue samples and ensures that agonal physiological changes which may confound research are avoided. Anticipating the impact of the experimental effects will often allow suitable endpoints to be identified. In cases where the experimental effects are unknown, it may be appropriate to conduct pilot experiments in which the animals are intensively monitored. Conducting a literature review, discussing ideas with colleagues and consulting CCMP staff can assist you in defining

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Pain and Distress in Lab Animals, cont. from pg. 3

TABLE 2: APPEARANCE
Ungroomed
Piloerection
Abnormal stance
Hunched
Red tears = porphyrin secretions (rats only)
Eyelids half shut
Dilated pupils
Nasal discharge
Recumbent

TABLE 3: BEHAVIOR
↓ activity
↓ appetite
↓ drinking
Guarding limbs
Self-mutilation
Aggression
Vocalization
Aversion towards cage mates
Delayed or exaggerated response to stimulation

NEW BAR CODE CAGE IDENTIFICATION SYSTEM IMPLEMENTED

A new cage identification system was recently introduced employing a scannable bar code to identify and count individual cages. The system will enhance the efficiency and accuracy of weekly cage counts performed by RARC's Husbandry and Operations staff. All existing cage cards are now identified with an individualized label containing a unique bar code and the name of the Principal Investigator (PI). When scanned, individual cages will be counted and tabulated for recharge. As each bar code is unique, double counting cannot occur.

Many laboratories use the cage card for recording important strain and experimental information. RARC staff worked with individual laboratories to find space on the cage cards for the bar code label.

Importantly, laboratories will need to apply a bar code label, specific for the PI to be charged, when new cages are created as a result of weaning or when new experimental groups are created. A process for requesting labels, in advance, is available. Laboratory staff must still record all newly created cages as well as those removed from the holding room on the *Daily Census Sheet*. Future enhancements to the system are planned.

objectives, identifying parameters to be monitored, determining the best study design, as well as selection of the most appropriate animal model. Results from previous studies may also help you identify expected clinical signs, their onset and duration.

Changes in body weight, as well as the animal's physical appearance, clinical signs and behavior (natural and provoked) can serve individually or collectively as indicators of pain and distress and used to define a humane endpoint. Body weight changes may reflect a reduced rate of weight gain in growing animals or actual weight loss. Body weight decreases are often accompanied by a decrease in food and water consumption. As a rule of thumb, rodents losing more than 20% of their body weight should be removed from the study or euthanized. However, sometimes body weight can be difficult to assess accurately, particularly where tumors, pregnancy or organomegaly are involved. These conditions may mask overall body weight loss due to dehydration, fat loss, muscle atrophy/wasting and therefore body condition scoring (BCS) is a useful tool to monitor health status. Body conditioning scoring is achieved by palpating the vertebral column and dorsal pelvic bones of the mouse and assigning a score as illustrated in Figure 1 based on the prominence of the bones and the relative fleshy covering of the skeleton.

Subtle changes in a rodent's physical appearance may also give insight into their physiologic state. Table 2 highlights some of the more common changes that may be observed in unhealthy rodents.

Other parameters indicative of an altered physiological state include: elevated blood pressure/heart rate, disrupted sleep patterns, body temperature, and rapid shallow breathing. Acute phase proteins (APPs) such as serum amyloid A in the mouse and α2-macroglobulin in the rat can serve as indicators of infectious disease severity and progression. Fever is a common response to infection and is often transient especially in rodent models. Hypothermia can be a strong indicator of deterioration. Body temperature decreases have been shown to be effective indicators of imminent death in infectious disease models. As a rule of thumb, in rodents decreases of 4-6˚C body temperature is used as a surrogate endpoint to death.

Ideally animals should be observed during their active period and from a distance to determine behavioral changes (See Table 3). Rats and mice are nocturnal and therefore daytime observation of sleeping patterns, appearance, posture, grooming patterns, and activity levels should be recorded.

In summary, death or extreme pain/distress should be avoided as experimental endpoints. Signs often exist that death may be a predictable outcome. An animal unable to reach or eat their food or water is at risk of starvation and dehydration and therefore death. An animal that is obviously moribund, e.g., convulsing or in persistent recumbency, should be removed from a study and euthanized. The earliest endpoint that is consistent with the scientific objectives of the study should be defined in the animal use protocol. Methods of pain/distress minimization that are consistent with the scientific objectives should be employed. The duration of pain/distress should be minimized. Consider conducting pilot studies to determine morbidity, expected timelines and responses, as well as the frequency of observation necessary. Observation frequencies and responsible staff should be identified in advance. Ideally, endpoint determination should be a team decision involving the PI, veterinarian, animal care/technical staff and the IACUC. Members of the research team should be trained to recognize normal/abnormal animal behavior, appearance, physiology, clinical signs associated with the experiment. The monitoring frequency should be sufficient to detect changes to prevent unrelieved pain or distress and should increase as clinical signs progress. Documentation of the onset, duration and severity of changes also helps to identify patterns and progression.

Animals experience pain and distress in a way that is comparable to human suffering and we, as animal users, have both a duty to protect their welfare as well as promote the practice of sound science. The establishment of humane endpoints allows us to strike a balance between these two responsibilities.

Additional reading:

1. "Humane Endpoints for Animals Used in Biomedical Research and Testing". ILAR 2000 Volume 41, Number 2.
2. W.M.S. Russell and R.L. Burch. The Principles of Humane Experimental Technique. 1959 http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc.
3 "Recognition and Alleviation of Pain in Laboratory Animals". ILAR 2009
4. Foltz CJ, Ullman-Cullere M. Guidelines for assessing the health and condition of mice. Lab Animal. 1999; 28 (4): 28-32.

IVF Recovery Success Levels

Measured by the percentage of 2-cell stage embryo obtained in IVF

Excellent (result above 66%) - Strain can be recovered in abundant quantities with extra flexibility to quickly build a colony.

Good (41-65%) - Strain can be recovered in abundant quantities to build a colony.

Fair (20-40%) - Strain can be recovered in sufficient quantities to build a colony.

Quality Control to Live Born (QCL) is recommended (10-20%) – Strain may be at risk. It should be recovered to live born to determine the quality of the frozen sperm and evaluate what work will be required and what costs above standard, incurred to recover and build a colony.

Freeze Backup is recommended (below 10%) - Strains producing less than 10% 2-cells may be challenging to recover by standard IVF methods. Consider repeating the cryopreservation with new males.

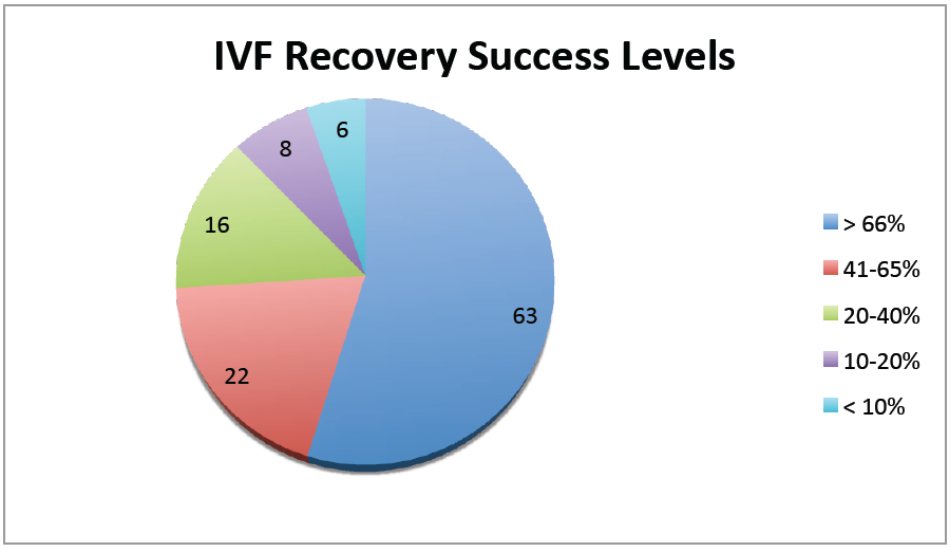
Cryobanking

Continued from page 1

percentage of frozen samples showing good recovery upon thaw test evaluation. As explained in the inset to the left and illustrated in the figure below, strain recovery was excellent for 63 strains (55% of the total number of the frozen strains), good for 22 strains (19%) and fair for 16 strains (14%) as measured by development of embryos to the 2-cell stage upon IVF using frozen sperm. Recovery to live born pups following IVF was recommended for only 8 strains (7%) and only 6 strains (5%) possibly requiring a second trial of freezing.

While this project will allow WCMC to reduce mouse space usage, cryopreservation also saves money. The cost of cryopreserving a mouse line is less than maintaining a live colony for six months. Moreover, cryobanking can prevent genetic drift in a breeding colony. Therefore, the MGCF recommends investigators freeze their GEM strains as soon as the mice are deemed useful for their research purposes. The MGCF has been performing sperm cryopreservation for over 10 years. Weill Cornell investigators interested in cryopreserving GEM strains should contact the MGCF.

[1]Ostermeier, G., Wiles, M.V., Farley, J.S., and Taft, R.A. (2008) Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. *PLoS ONE* 3(7): e2792



"Age of Enforcement" Announced

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management, or production efficiency.

Negative public opinion surrounding the use of animals in research has become increasingly more widespread since the AWA was passed in 1966. This has put pressure on the USDA to ensure it is "enforcing the AWA to the fullest extent possible" (USDA News Release May 2010. *APHIS' Enhanced Animal Welfare Act Enforcement Plan*). Recently the Office of the Inspector General (OIG) audited the USDA section which implements the AWA. The resulting deficiencies required the USDA to develop an action plan, whereby they improve their record of enforcement of the AWA regulations. In short, the focus of the action plan is to shift from an education focus to an enforcement focus as well as improve inspector performance and regulatory compliance. New for 2010, the USDA has developed an "Inspection Requirements" guideline for their inspectors which outline a consistent methodology for inspections. Under the list of 'intermediate' actions, to be completed within 6 months, are the requirement to improve citation and documentation of violations leading to stronger investigations and increasing the likelihood of successful enforcement actions. The USDA will also establish new increased penalties for AWA infractions. In the short term, the USDA will make public violations of the AWA, improve supervision of inspectors and improve the quality of inspections.

How does the USDA's "Age of Enforcement" impact us?

RARC is consistently striving to mitigate regulatory risk for our institution as regulatory non-compliance can lead to significant public relations issues which jeopardize the institution's mission and can lead to penalties and fines. What does this mean to scientists? Unfortunately, but necessarily, more compliance monitoring and internal audits. Please recognize that this process has been implemented to protect your research interests.

Education & Training at the Research Animal Resource Center

2011 TRAINING SESSIONS
January-March

RARC Orientation
Tuesday, Jan. 4, 2:00pm-4:00pm
Thurs., Jan. 20,10:00am-12:00pm
Tuesday, Feb. 1, 2:00pm-4:00pm
Thurs, Feb. 17, 10:00am-12:00pm
Tuesday, Mar. 1, 2:00pm-4:00pm
Thurs., Mar. 17, 10:00am-12:00pm

Xenograft Training
Monday, Jan. 24, 2:00pm-3:00p
Monday, Feb. 28, 2:00pm-3:00
Monday, Mar. 21, 2:00pm-3:00pm

Hazardous Materials Training
Weds., Jan. 26, 2:00pm-3:00pm
Weds., Feb. 23, 2:00pm-3:00pm
Weds., Mar. 23, 2:00pm-3:00pm

Rodent Surgery
Wet Lab sessions:
Fri., Jan. 7, 10:00-11:30
Fri., Feb. 11, 10:00-11:30
Fri., Mar. 4, 10:00-11:30
Lecture sessions:
Tues., Jan. 18, 10:30-11:30,
Tues., Feb. 15, 10:30-11:30,
Mon., March 21, 10:30-11:30,

Rodent Breeding:
Weds., Feb. 23, 10:00-11:30am

RARC's education and training program provides information on all aspects of the use of animals in research, education and testing as well as informs biomedical investigators of the rules and regulations governing their use. The Education and Quality Assurance (EQA) staff are available to provide hands-on wet labs and special procedure training and a wide variety of other services useful to the biomedical investigator. EQA publishes a RARC "User's Guide" annually, which is available as a hard copy or on CD. A variety of animal care and use guidelines and policies are accessible over the intranet or from our main offices.

EDUCATIONAL SESSIONS AVAILABLE:

NEW INVESTIGATOR ORIENTATION- Offered Twice a Month This session is required for all investigative staff regardless of their role in the animal use project and is designed to introduce new and experienced staff to the use of animals in biomedical research in general and the services and facilities of the Research Animal Resource Center in specific.

RODENT SURVIVAL SURGERY TRAINING- Offered Twice a Month All investigators conducting rodent surgery are required to attend at least one of the sessions offered prior to performing surgical procedures on rodents regardless of previous animal surgical experience. The following areas are covered in each session - aseptic technique, surgical equipment and instrumentation, animal and surgeon sterile preparation, suture material and wound closure, postoperative care including pain management and record keeping requirements. The hands-on session is strongly recommended for all individuals especially the novice as it provides a real-life experience; the didactic session is appropriate for personnel at all levels of experience. All investigators are welcomed to attend both sessions.

HAZARDOUS MATERIAL TRAINING- Each Session is Offered Monthly. Investigators conducting studies in animals treated with hazardous chemicals, carcinogens, radionuclides or biological agents are required to attend all applicable haz-mat room training prior to beginning this work. Each training session addresses compliance with OSHA's Bloodborne Pathogen Standard and MSKCC/ WCMC's Laboratory Safety training as well as all applicable RARC guidelines developed for the use of select agents. All personnel working with hazardous material in animals must attend all applicable sessions:

1. - Hazardous Materials Suite(s) Training
2. - Xenograft Room Training
3. - Isotope Room Training (also requires WCMC/MSKCC Radiation Safety Training)

ADDITIONAL TRAINING- To facilitate the needs of our investigators, advanced training sessions are available by appointment Please contact the EQA Administrator for scheduling and/or to discuss your needs.

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