

# Mouse Breeding Colony Management



**Research**

*Division of Laboratory  
Animal Resources*



# Mouse Breeding Colony Management

## Why do I Need A Breeding Colony

- To produce a new line, strain, or expand an existing colony?
- To maintain a line?
- To supply animals for research?
- To provide animals for experimental use
- To provide enough animals for replacement breeders
- To keep the colony viable



# Mouse Breeding Colony Management

## Why Mice?

- Mice comprise the majority of all research animals
- They are genetically similar to humans
- They mature quickly and can advance in generations quickly
- They are inexpensive to maintain



# Mouse Breeding Colony Management

## Why Mice?

- They have a short life span and can be studied throughout their entire life cycle
- They are small and easy to house
- They are easy to breed



# Mouse Breeding Colony Management

FVB



Albino coat color

Adults > 30 grams

Aggressive

Average breeders

Good mothers

Large litters

9.5 pups/litter  
average (JAX)

# Mouse Breeding Colony Management

## C57BL/6

Black coat color

Adults 25-30 grams

Developed by C.C. Little ~ 1921

### Health:

Eye defects

Malocclusion

Dystocia

UD (ulcerative dermatitis)

Hydrocephalic (JAX)

Commonly used as an inbred background strain

### Average breeders:

6.6 pups/litter & 87% weaned (JAX)



# Mouse Breeding Colony Management

129



Several sub-strains range in color from white to chinchilla or agouti (129SvEv Taconic)

Docile

Source of ES cells

Variable breeders (genotype dependent)

129/J: 4.6 pups/litter

129/SvJ: 6.0 pups/litter

# Mouse Breeding Colony Management

## Know Mouse Life/Reproductive Data

- Biological life span ~ 2 years
  
- Basic life stages:
  - Neonate: birth to wean (21d)
  - Sexual maturity: ~6 weeks
  - Adult size: 8 - 10 weeks
  - Geriatric: 18 months



# Mouse Breeding Colony Management

## Know Mouse Life/Reproductive Data

- ❑ Optimal breeding window is much shorter
  - ❑ Female ~ 9 months (approx. 6 litters)
  - ❑ Male ~ 1 year
- ❑ Average estrous cycle – every 4-5d lasting up to 10hrs
  - ❑ Signs of estrus include:
    - ❑ Swollen vulvar area
    - ❑ Redness at vulva



**Post-partum estrus –up to 20 hours after parturition**

# Mouse Breeding Colony Management

## Know Mouse Life/Reproductive Data

- Weaning age - 20-21 days. Longer 21 days requires approval from the IACUC
  - Some breeding information can be dependent on genotype:

<u>Strain</u>	<u>Performance</u>	<u>Avg. Litter Size</u>
CD-1 (outbred)	Excellent	7+
B6C3F1 (hybrid)	Excellent	7+
DBA/2	Poor	3-4
Tg/KO	?	?

# Mouse Breeding Colony Management

## Setting Goals For The Colony!



# Mouse Breeding Colony Management

## Three “R”s of Research

**REDUCE** the number of animals in research (or excessive production)

**REFINE** experiments to minimize pain, distress, or unnecessary production

**REPLACE** animal use with computer models or in vitro research



# Mouse Breeding Colony Management

## What is the Goal of the Project

- Number of mice needed per week or month to meet experimental needs
- Continuous vs Intermittent production
- Can you use Males and Females or only one sex
- How often can you expect a litter
- How many pups of the appropriate genotype can you expect per litter
- How often will you have to replace breeders
- Is a Genetic Profile needed
- How long will the breeding colony be maintained
- What will happen to the mice when the project is completed

# Mouse Breeding Colony Management

## How to Improve Studies with Colony Goals

**Maximize output:** per cage, per unit area, and per unit of labor by selecting breeders properly and by replacing unproductive breeders

**Maximize quality:** health, genetic authenticity, and uniformity of offspring

**Equate supply with demand:** low wastage and flexibility of production



# Mouse Breeding Colony Management

## Cost of Poor Breeding and Colony Management

- ❑ Lost experiment time due to lack of mice
- ❑ Lost per diem and materials for unproductive cages
- ❑ Increased expenses if average litter size is not optimized
- ❑ The most common mistake is the **failure to notice**
  - ❑ individual mouse deaths, problems, performance
  - ❑ individual cages-- breeding problems, production drops, age to retire
  - ❑ entire colony trends of problems, performance (or lack of performance)

# Mouse Breeding Colony Management

## Ways to Organize your Colony & Projects



- Use different and **consistent** color of cage card for each strain
- Physically separate similar strains (names, coat colors, etc.) as much as possible
- Use correct nomenclature and identification on every card
- Keep older cards in the cage card holder until cage is eliminated from the colony
- Identify mice by physical ID (ear punch, tag, etc.)

# Mouse Breeding Colony Management

## Organize your Colony & Projects

Identify Strains or Projects





# Mouse Breeding Colony Management

## Information for Breeding Cage Cards

Breed and strain

Ancestry

Animal ID #s

Sex

Date mated

Date of birth with number of pups

Date weaned with sex and number of weanlings

Veterinary information

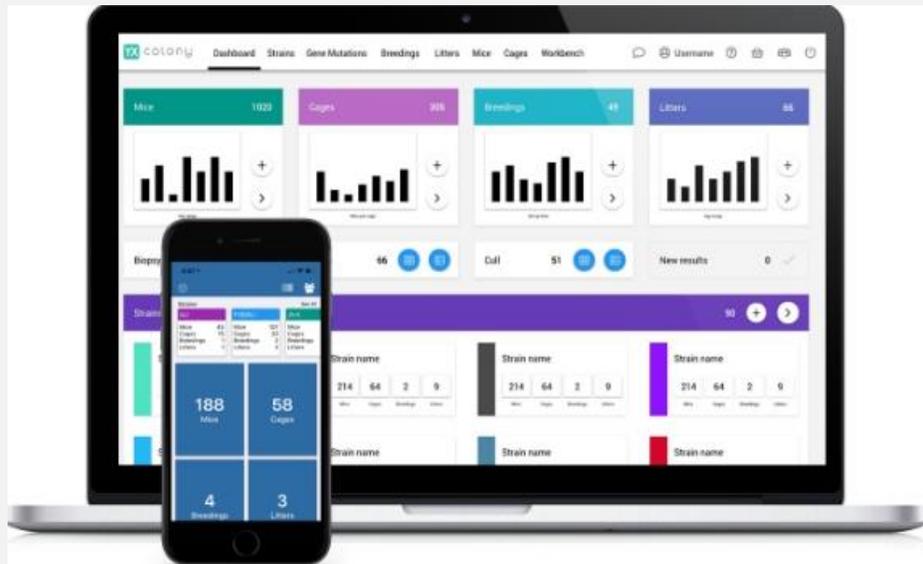
Female		Male	
ID#	DOB	ID#	DOB
9	10/14/02	3	10/14/02
Genotype 4 ko/ko 8		Genotype 2 m2/ko 8	

Date Born	# Born	Date Weaned	Females Weaned	Males Weaned	Comments
4/10	8	5.1	2	6	F3
5.1	7	5.20	3	5	lost pups
5.22					

# Mouse Breeding Colony Management

## Record-Keeping



### Types of Records:

- Physical identification
  - Cage cards
  - Individual animal
- Electronic database
  - Transnetyx “AMI”
  - SCION from Topaz
  - MS Excel spreadsheet

# Mouse Breeding Colony Management

## Record-Keeping

Accurate and current records are the most important factors in an efficient breeding program

- Aids troubleshooting
- Saves time
- Improves organization
- Facilitates compliance with divisional, university, local, state, and federal guidelines and laws

# Mouse Breeding Colony Management

## Record-Keeping- Monitoring

- Individual animals, their ancestors, siblings and descendants
- Matings between animals
- Litters born from such matings, and the individuals within litters that are used experimentally or for the next generation of breeding
- Experimental material (tissues and DNA samples) obtained from individual animals
- Test results from the samples

# Mouse Breeding Colony Management

## Record-Keeping- Animal Identification



Ear Punch or Notch



Tattoo

# Mouse Breeding Colony Management

## Record-Keeping- Animal Identification



- Microchips are durable ID
- They require the use of scanners to read
- They can be used to measure other bodily functions such as heart rate, temperature, etc.
- They can be expensive compared to tattoo or ear punching

# Mouse Breeding Colony Management

## Select the Proper Breeding System

Intensive Pairs or Trios  
Non-intensive Breeding System  
Timed Matings



# Mouse Breeding Colony Management

## Select the Proper Breeding System

### Intensive Breeding System

Mate one male with one female in the same cage for their entire reproductive life

#### ADVANTAGES

- Takes advantage of the post-partum estrus for faster turn around on litters.
- Easier to keep track of mice- they aren't being moved into other cages frequently.



#### DISADVANTAGES

- High demand for space, cages, and equipment
- Supports more males than are actually required for production
- Must wean older litter before second litter is born or risk death of newborns
- Risks aggression from male towards the pups

# Mouse Breeding Colony Management

## Select the Proper Breeding System

Non-intensive breeding system

Females are placed in the male's cage only for breeding and then separated

### **ADVANTAGES**

- Reduced risk of fighting between aggressive females
- Males cannot kill the newborns
- Increased flexibility of production

### **DISADVANTAGES**

- High Labor costs
- Lost access to postpartum estrus
- Requires more observation of moms to identify mice that are poor producers verses mice that are “forgotten” by technician

# Mouse Breeding Colony Management

## Select the Proper Breeding System

### Timed Matings

Used when you need to know exact day of gestation to collect embryos or forecast delivery date

Females are added to males cage then checked each morning for “plugs” to confirm mating and approximate time of mating as the middle of the preceding night

# Mouse Breeding Colony Management

## Select the Proper Breeding System

### Timed Matings

When plug is found, that is “DAY 1/2” (calculate that breeding actually took place at midnight / early morning that day)

What is a “plug”?

- The ejaculate from the male's accessory sex glands forms a short-lived, white to yellowish plug in the vagina of the female

# Mouse Breeding Colony Management

## Select the Proper Breeding System

### Vaginal Plug

Presence of a plug does not guarantee a mouse is pregnant

- 15% or more of plugged females are either not pregnant or never gestate due to reabsorption of fetuses.

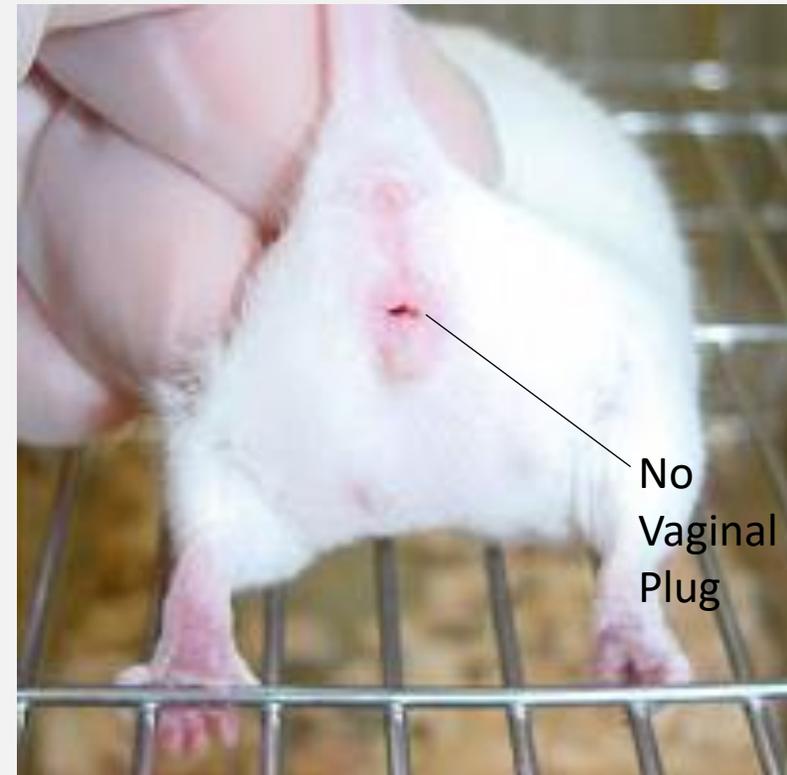
Absence of a plug does not guarantee a mouse is not pregnant

- The plug may have dissolved before you saw it, or may have been too deep, too small etc.

# Mouse Breeding Colony Management

## Select the Proper Breeding System

### Vaginal Plug



# Mouse Breeding Colony Management

## Select the Proper Breeding System

### Vaginal Plug

#### Restraint

- on wire lid-easiest
- Scruff

#### Tools

- forceps
- toothpick
- probe
- fingers



# Mouse Breeding Colony Management

## Gestation & Detection of Pregnancy

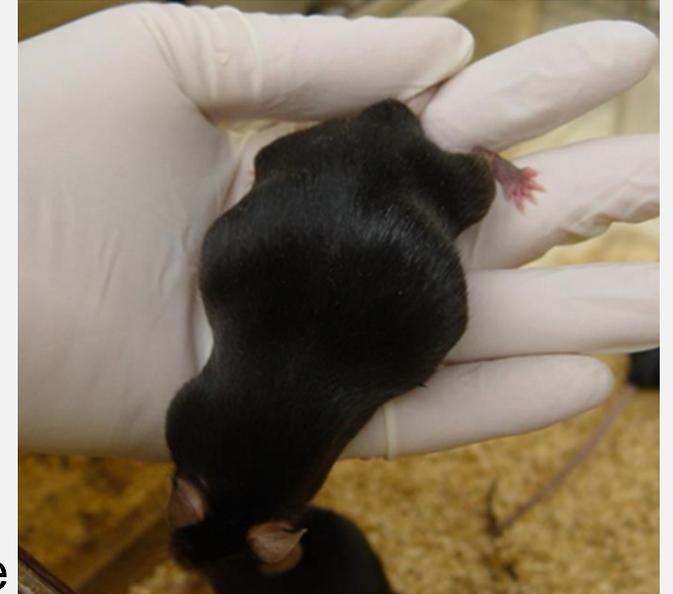
Mouse gestation is 19-21 days.

In absence of plug check, detection of pregnancy is hit or miss.

Observation

Parturition

Palpation (about 14 days, may detect what feels like a string of pearls)

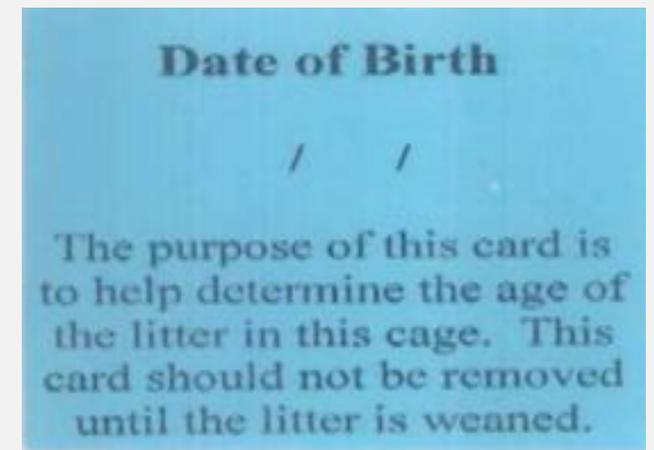


**Not Recommended- may stress mother out or inadvertently rupture something inside.**

# Mouse Breeding Colony Management

## Parturition

- ❑ Giving birth at the natural end of gestation
- ❑ Best chance of litter survival when female is left undisturbed for 2-3 days before and 4-5 days after parturition
- ❑ Arrange for cage to be changed beforehand or left unchanged during this period if sensitive strain



Reduce handling and observations to a minimum

# Mouse Breeding Colony Management

## Postnatal Development & Weaning

Recognizing developmental stages helps determine how old pups are

Weaning on time prevents doubling up of litters and trampling to death of new litters

- Timely weaning also prevents “Overcrowded Cage” charges

Normal v. Abnormal Development:

- runts
- malocclusion



# Mouse Breeding Colony Management

## Age Determination

Day 1: Blood red in color and hairless. Very small, no milk spot. Eyes and ears are closed



Day 2: Dark pink, Milk spot is present, Eyes and ears closed

Day 3: Pink, Slightly larger, milk spot is bigger and more opaque. Ear nubs are apparent

Day 4: Pink. Ears stand out from head



# Mouse Breeding Colony Management

## Age Determination

Day 5: 'Stubble' or 'dander' on back, early signs of pigment (on dark furred mice) Milk spot fading



Day 6: fur and pigment starting to show. Ears more prominent and detailed

Day 7: Bigger. Fur and pigment filling in



Days 8-10: Feet look more formed. Inner ears look fully developed. Eyes are still closed most of the time. Fur and pigment fully established

# Mouse Breeding Colony Management

## Age Determination

Days 12-14: eyes are open. Pups are mobile and may seem hyper or jumpy due to auditory development



Days 14-16: begins to eat solid food. Looks anatomically formed but slightly juvenile in posture and attitude



Days 17-21: Fully active. Looks like a complete (but small) adult. Ready for weaning at 20-21 days old

# Mouse Breeding Colony Management

## Weaning

**IT IS VERY IMPORTANT TO WEAN ON TIME!**

Timely Weaning prevents **Trampling Deaths** and overcrowded charges!

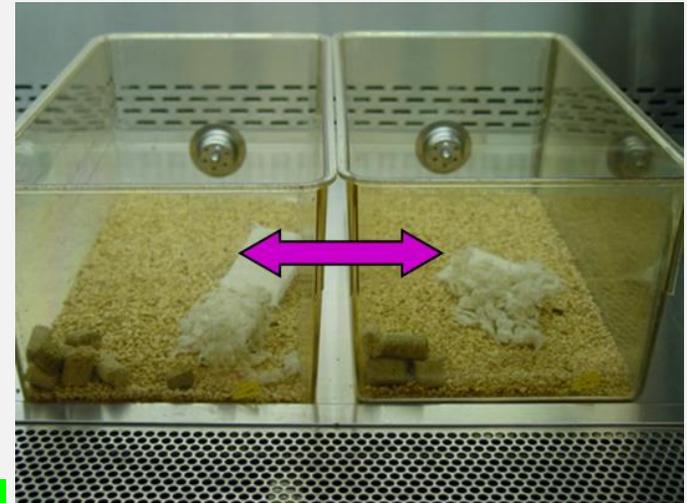
Note size of 21-day old pups and if mother is pregnant again in your records

# Mouse Breeding Colony Management

## Weaning

### To Prepare Weanling Cages:

- Carry over some dirty bedding and nestlet from parent cage
- Put 5-6 pellets of food on cage floor
- Be sure wire bar is adequately filled with **feed**
- Be sure the cage has a **water bottle** with a tight lid to prevent leaks



# Mouse Breeding Colony Management

## Weaning

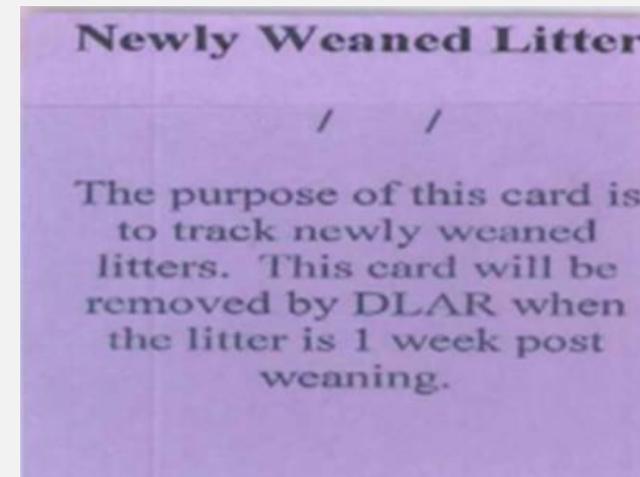
### To Prepare Weanling Cages:

- Separate weanlings by sex-  
See:
  - [DLAR Mouse Housing Density Policy](#)
  - [DLAR Housing Information](#)
  - [IACUC Policy 110](#)
  
- Label cage with a purple or blue “Newly Weaned Litter” Card



Sexing 21 day old pups:

- Anogenital distance
- Presence of nipples



# Mouse Breeding Colony Management

## DLAR Housing Density Policy

**One** Litter per cage MAX!! More than one will result in an investigator attention.

There should be no more than 2 adults in the cage while a litter is present.

If you are going to use an “auntie” mouse she should either not be pregnant or removed before her litter is born, otherwise you will receive an Investigator Attention Notification for non-compliance.



# Mouse Breeding Colony Management

## DLAR Housing Density Policy

Litters should be weaned at 21 days if the male is left in the cage after mating.

If the male is removed after mating, you may have 28 days to wean the litter.

**Males should never be split and then recombined- this will result in fighting behavior.**

# Mouse Breeding Colony Management

## DLAR Housing Density Policy

If you want to have the 28-day weaning date, the male needs to be removed before the litter is born. As stated earlier, mouse gestation lasts about 21 days, and postpartum estrous can start immediately-24hours after the birth of a litter. If the female mouse gets pregnant directly after giving birth because the male is still in the cage, then her current litter will have to be weaned at 21 days or you will risk having an investigator attention when the female gives birth to the second litter.

# Mouse Breeding Colony Management

## Weaning Index

Calculate a weaning index for each breeding pair and for each strain, To estimate and track production by averaging the number of pups weaned per month per female

This number can vary considerably depending on the strain that you are working with

The lower the index, the more time and resources will be needed to reach your goal



# Mouse Breeding Colony Management

## Calculating a Weaning Index

Total number of pups weaned divided by the number of times she has been mated

**Example: 20 (pups weaned) ÷ 5 (times mated)  
= 4 pups per month**

# Mouse Breeding Colony Management

## Factors Influencing the Weaning Index

- Some induced mutations cause low production or poor viability (failure to thrive) or genetic lethality
- Poor breeder productivity, pre-weaning deaths, cannibalism, still-born pups
- Delayed implantation or environmental incidents

# Mouse Breeding Colony Management

## Factors Influencing the Weaning Index

- Long gaps between litters
- Harem breeding requires separation from males eliminating post-partum breeding opportunity
- Age of males and females, the older they are the less productive

# Mouse Breeding Colony Management

## Know Mouse Behavior

- ❑ Gnawing mammals (rodents)
- ❑ Omnivorous, semi-continuous feeders
- ❑ Nocturnal, secretive & nesting
- ❑ Thigmotactic
- ❑ Social & communal with male-dominated hierarchy
  - ❑ Males will fight: injuries & fatalities possible.
- ❑ Communicate via pheromones



# Mouse Breeding Colony Management

## Breeding Tips

- Keep rooms quiet and avoid handling cages as much as possible-- especially avoid loud or sudden impact noises such as loud conversation, equipment collision, or radios
- Minimize traffic in and out of the room
- Minimize any source of vibration to cage location
- Do not wear perfume, cologne, or other strong scents in the room

# Mouse Breeding Colony Management

## Breeding Tips

- Use clean gloved hand to handle mice and avoid scent transfer between cages. Keep gloves moistened with disinfectant
- Work gently, slowly, and quietly with problem breeders and use the same technique and technician for breeding work
- Do not change cage for 2 days before or 3 days after litter delivery

# Mouse Breeding Colony Management

## Breeding Tips

- ❑ If moms are scattering or killing the newborns, try removing the male before delivery

**PAY ATTENTION TO THE  
MICE & KEEP GOOD  
RECORDS**

# Mouse Breeding Colony Management

## TROUBLE SHOOTING

# Mouse Breeding Colony Management

## Breeding Tips-My Mice are not Breeding

- Check sex of mice in the cage (should be 1 male and 1 or 2 females)
- Check age of mice (retire at 6-9 mo.)
- Check environmental factors in the room (light cycle, noises, etc.)
- Try using a different male
- Are you sure they aren't breeding?

Try doing plug checks to see if the loss of pups is occurring after conception

- Replace the breeders with a younger pair

# Mouse Breeding Colony Management

## Breeding Tips-My Mice are not Breeding

- Mice may cannibalize litters when disturbed during the first few days after delivery. Do not handle or disturb nest for several days postpartum.
- Some mice will cannibalize only their first litter
- Make sure the room is quiet, and the cage location avoids unnecessary traffic..
- Move mice to a more secluded location in the housing room
- Try removing the male before delivery (non-intensive system)
- Foster pups if needed and replace that breeder

# Mouse Breeding Colony Management

## Retire and Replace

- Retire mice over 6-8 months old or after 5-6 litters
- Replace breeders that have produced 2 consecutive poor litters (quality or quantity)
- Replace males that have not produced a positive pregnancy after 3-6 weeks with a receptive, fertile female with a different male



# Mouse Breeding Colony Management

## Dystocia

Female is dilated and clearly stressed:

Remove deceased pups and foster surviving pups

Female has delivered a few pups (dead or alive) and stops for several hours, but is active and alert:

Observe to make sure delivers rest of pups (usually dead), wait 7-10 days, then re-mate

# Mouse Breeding Colony Management

## Dystocia

Heroics if needed:

Immediately resuscitate any viable pups

\*\*If valuable female euthanized or dies, immediately remove ovaries for ovary transplants to maintain gene pool\*\*

# Mouse Breeding Colony Management

## Dystocia

**Contact your area vet tech!  
The sooner a dystocia is  
addressed, the more likely a  
favorable outcome for mother  
and pups.**

# Mouse Breeding Colony Management

Sometimes  
Things Happen...



# Mouse Breeding Colony Management

## Who Lives In a Perfect World?



Mom Dies

Genetic or Physical  
Abnormalities Prevent Moms  
From Caring for Litters

Some Mice Are Just Bad Moms!

# Mouse Breeding Colony Management

A Time May Come When You Need to Foster a Litter



# Mouse Breeding Colony Management

## When DO I Foster?

When keeping the pups alive is crucial to the strain

When there is no hope, the mom can or will care for them

Foster early! Each hour spent without proper care is critical; especially to newborns

# Mouse Breeding Colony Management

## Who Should Foster?

Select a mom who has already had a normal size litter about the same age as the one to be fostered

For best results, select a litter that is no more than 48 hours older than the litter to be fostered

Ideal foster mom and litter will be of a different coat color as the foster pups for easy identification

# Mouse Breeding Colony Management

## Who Should Foster?

Select moms that have had 2-3 successful litters weaned already

B6CBAF1 strain make excellent foster moms



# Mouse Breeding Colony Management

## Keep Total Litter Size the Same

Remove as many of her own pups as you wish to replace with foster pups

Milk production will suffer if total litter size varies by more than 2 from the original number

Humanely Euthanize extra pups from mom's original litter

With clean, gloved hand mix foster pups with the foster mother's own pups in the nest



# Mouse Breeding Colony Management

## Leave Them Alone

Leave mom and litter in a secluded, quiet place and observe from a distance her acceptance of the new pups

Moms will usually clean and nurse the new arrivals within the hour, if they are going to accept them



Successful foster should be noted in the first 6-12 hours or try another mom

# Mouse Breeding Colony Management

## Reminders

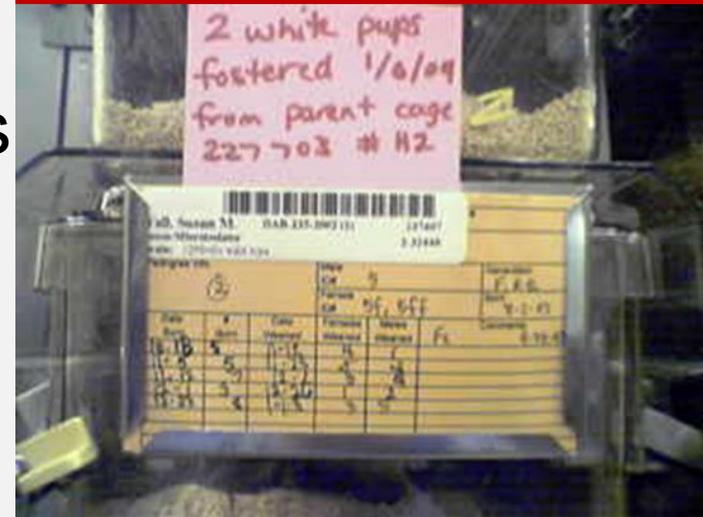
Make reminder notes of the ID on the cage card so you will know which are which at weaning

- BE SURE to correctly identify strain and pedigree of the fosters when time to wean!

Make similar notes on the birth mom's cage as well

- Note the success of the foster mom for use in the future

**You can never  
have too many  
reminders!**



# Mouse Breeding Colony Management

## How to Increase Litter Size

Litter size is often a trait specific to the strain

Selecting breeders for good performance may insure maximum production

Feed higher fat breeder chow  
(2918 = 6% fat; 2919 = 9 % fat)



# Mouse Breeding Colony Management

## How to Increase Litter Size

Intensive breeding system with 2 females will often increase overall success of the colony

Retire and replace lower than average breeders promptly to maximize colony production



# Mouse Breeding Colony Management

## Handling Unexpected Results

If your weanlings are not what you expected... wrong phenotype, wrong genotype, abnormally large litter size, or yield unexpected experimental results then you should

# Mouse Breeding Colony Management

## Handling Unexpected Results

Check your Breeding Colony Records

- Check the weanling card for the wrong ID or wrong parent information (match up parent and DOB info).
- Check the breeder card for the wrong ID (a homozygous cage? Back-crossed cage? Wrong strain name? Wrong selected color of cage card? Mixed-up cage cards?).
- Re-check the breeders genotype (strain impurity can result in greater than average litter size).
- Re-check your records for data on previous litters and any genotype testing on pups.

# Mouse Breeding Colony Management

## Handling Unexpected Results

**If That Does Not Solve The Problem, Then**

Suspect your Breeders Have a Problem

- You may need to eliminate the affected cages and start over

# Mouse Breeding Colony Management

## Dietary Management

### Harlan(ENVIGO) Global Rodent Diets



#### **2918 Non-Irradiated**

##### Key Features

- \* 18% Protein
- \* 6% Fat
- \* + Moderate phytoestrogen



#### **2919 Irradiated**

##### Key Features

- \* 19% Protein
- \* 9% Fat

**The irradiated  
version is used for  
Sterile Housing**

# Mouse Breeding Colony Management

## Dietary Management

### Weanlings May Need a Softened Diets

Dough Diet on  
Cage Floor



H<sub>2</sub>O DietGel  
76A



Moistened Food Pellets on  
Cage Floor

# QUESTIONS & SUPPORT

## VETERINARY SERVICE SUPPORT PERSONNEL

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