

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

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
- ❑ 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

FVB

Albino coat color
Adults > 30 grams
Aggressive
Average breeders
Good mothers
Large litters
9.5 pups/litter average
(JAX)



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)

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 Life Stage 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

Optimal Breeding Window

- ✓ Much shorter for Females
- ✓ Female ~ 9 months (approx. 6 litters)
- ✓ Male ~ 1 year

Weaning Age

- ✓ 18-21 days
- ✓ Longer than 21 days requires approval from the IACUC

Mouse Reproductive Data

Average estrous cycle

Every 4-5d lasting up to 10hrs

Signs of estrus include:

- ✓ Swollen vulvar area
- ✓ Redness at vulva

Post-partum estrus

May last up to 20 hours after parturition



Breeding Information for Different Genotypes

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	?	?

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

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

Improve your Research 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

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

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)

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.)



Ways to Organize Your Colony & Projects

Cage Card

Identify Strains or Projects



Breeding Cage Card Information

Dingledine, Raymo DAR-069-2000- (1) 178232
 Mouse-Microisolator 4/19/03 5-26444
 Strain: mGlur 4 X mGlur 8

Protocol #
 Mating Date
 Mating #

Female ID#	9	$\left\{ \begin{array}{l} 1 \\ 2 \end{array} \right.$	DOB	10/14/02	Genotype	4 ko/ko 8
Male ID#	3		DOB		Genotype	2 h2/ko 8
Date Born	# Born	Date Weaned	Females Weaned	Males Weaned	Comments	
4/10	8	5.1	2	6	F3 3/19	
5.1	7	5.20	3	5	test pups	
5.22						

breeding card

Breed and strain

Ancestry

Animal ID #s

Sex

Date mated

Date of birth with number of pups

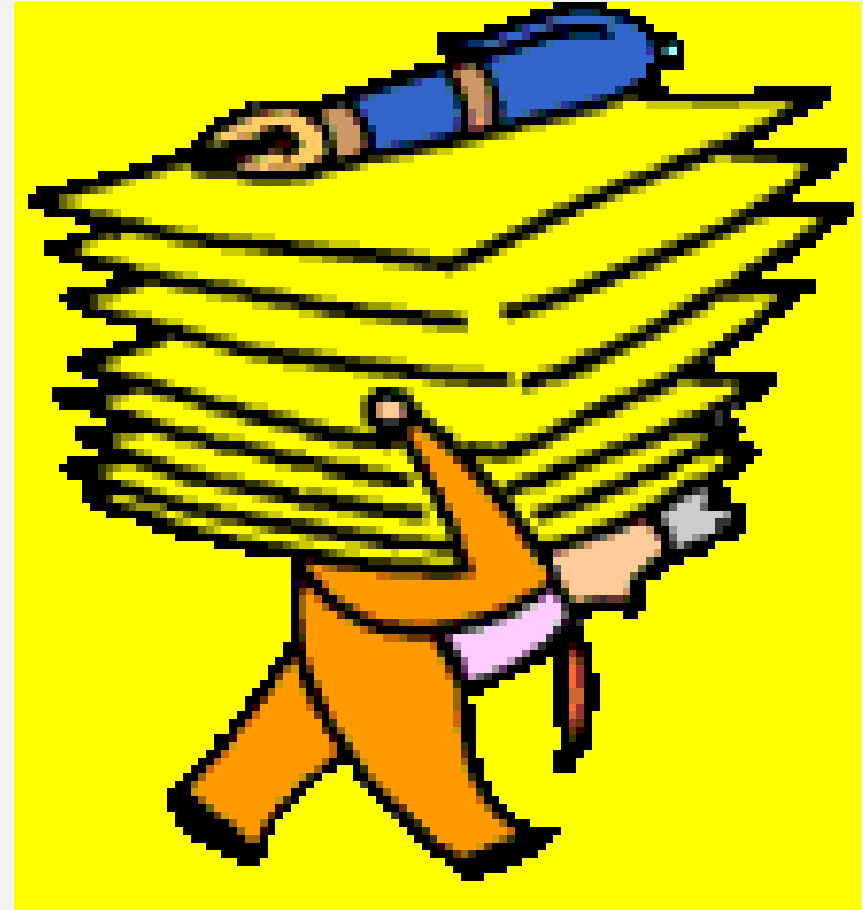
Date weaned with sex and number of weanlings

Veterinary information

Record-Keeping

Types of Records:

- Physical identification
 - Cage cards
 - Individual animal
- Electronic database
 - SCION from Topaz
 - MS Excel spreadsheet



Why Accurate Record-Keeping

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



To monitor

- ✓ 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

Animal Identification Methods



Ear Punch or Notch



Tattoo



Ear Tag



Animal Identification Methods

Microchip

- ✓ 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



Breeding System



- ✓ Intensive pairs or trios
- ✓ Non-intensive breeding system
- ✓ Timed matings

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
- ✓ 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
- ✓ If moms are scattering or killing the newborns, try removing the male before delivery
- ✓ **PAY ATTENTION to the mice and keep good records**

Intensive Breeding System



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

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

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.

Non-intensive Breeding System

Females are placed in the male's cage only for breeding and then separated- MALES ARE NEVER RECOMBINED INTO HOME GAGE.

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

ADVANTAGES

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

Timed Mating Breeding System

- ✓ 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
- ✓ 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

Visually Checking for 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.



Checking for Vaginal Plug with Tools

Restraint

- ✓ On wire lid- easiest
- ✓ Scruff

Tools

- ✓ Forceps
- ✓ Toothpick
- ✓ Probe
- ✓ Fingers



Gestation & Detection of Pregnancy

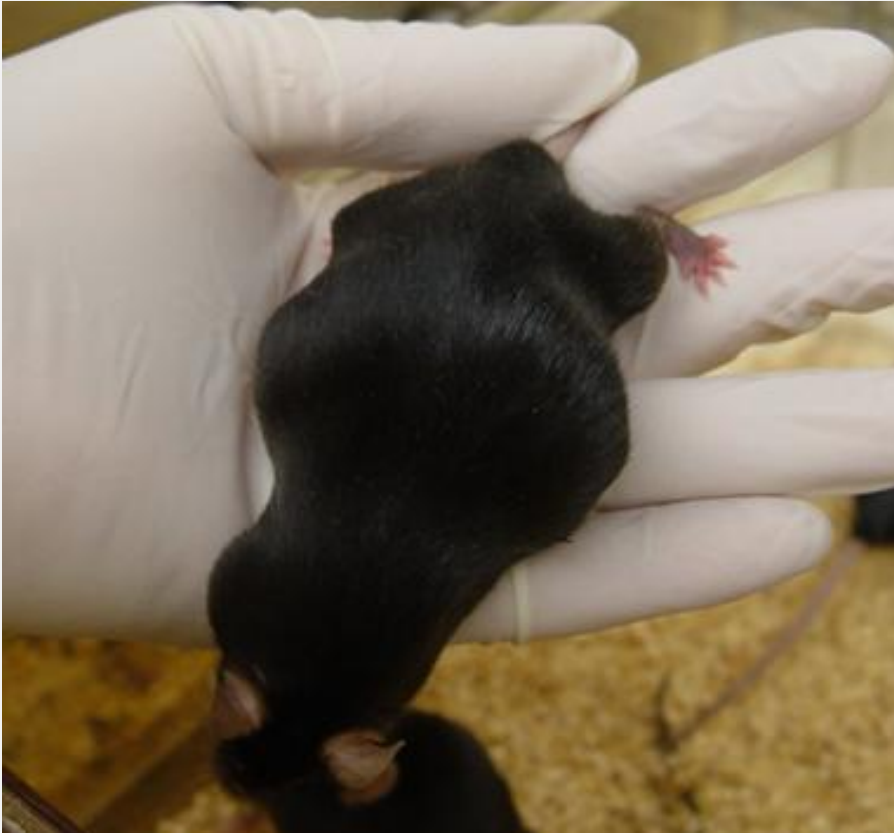
Mouse gestation is 19-21 days.

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

- ✓ Observation
- ✓ Parturition
- ✓ Palpation

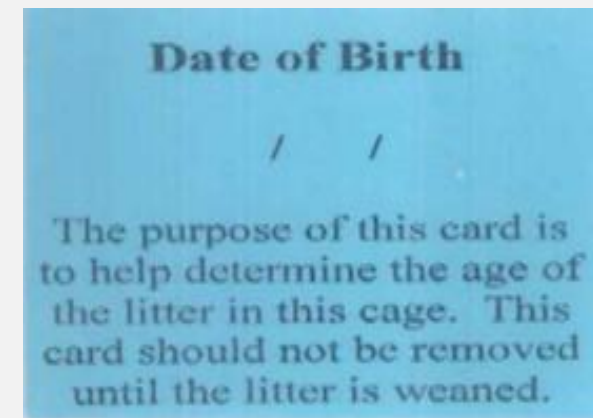
At about 14 days, may detect what feels like a string of pearls

- ✓ Not recommended
- ✓ may stress mother out or
- ✓ inadvertently rupture something inside



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



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 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

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

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

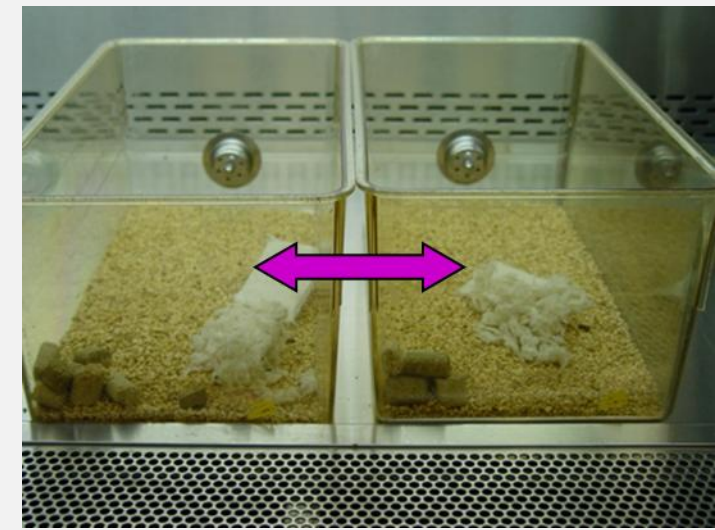
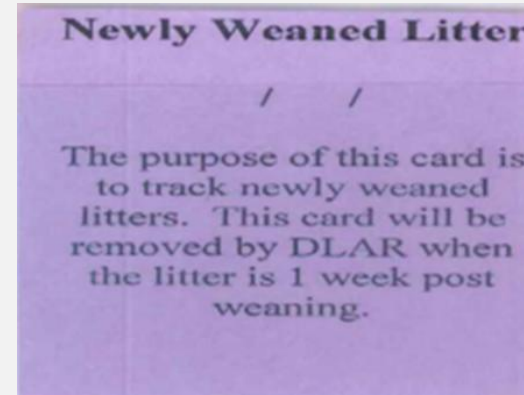
Days 17-21: Fully active. Looks like a complete (but small) adult. Ready for weaning at 20-21 days old

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

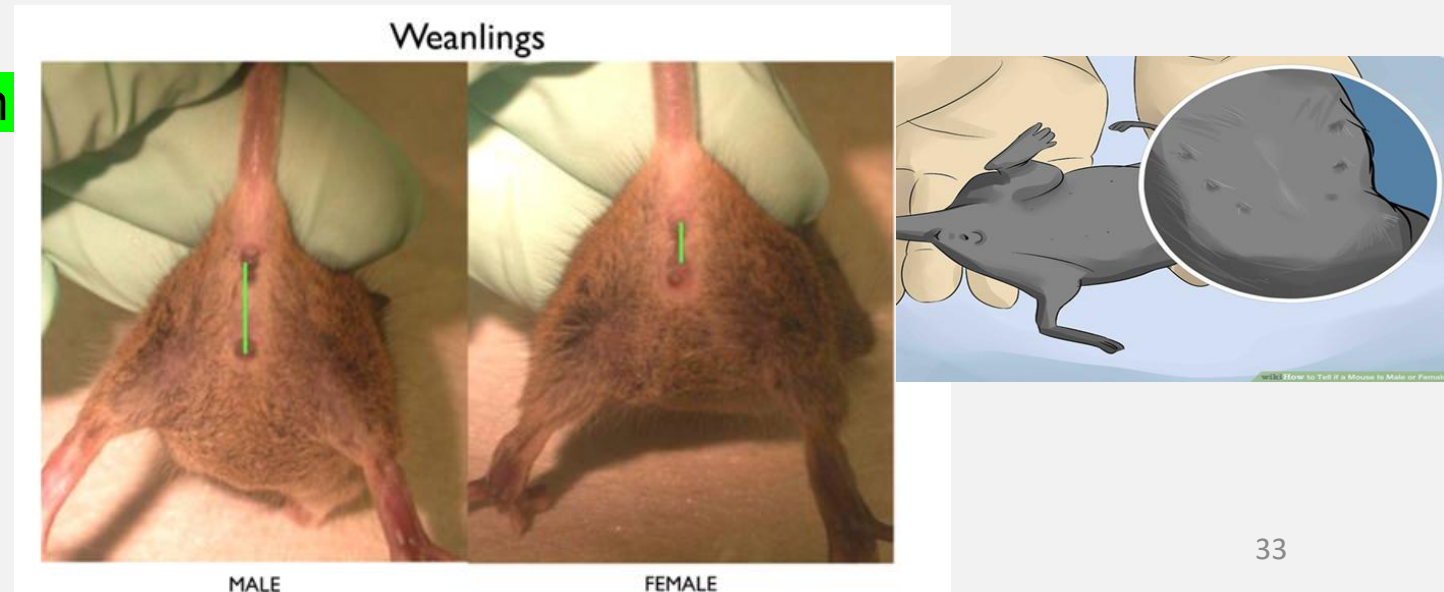
- ✓ To Prepare Weanling Cages:

- ✓ 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 tight lid to prevent leaks
- ✓ 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



Facility Rules

- ✓ 1 Litter per cage MAX!! More than one will result in an investigator attention.
- ✓ 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.
- ✓ There should be no more than 2 adults in the cage while a litter is present.
- ✓ Males should never be split and then recombined- this will result in fighting behavior.

Facility Rules Cont.

- 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.
- 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.

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

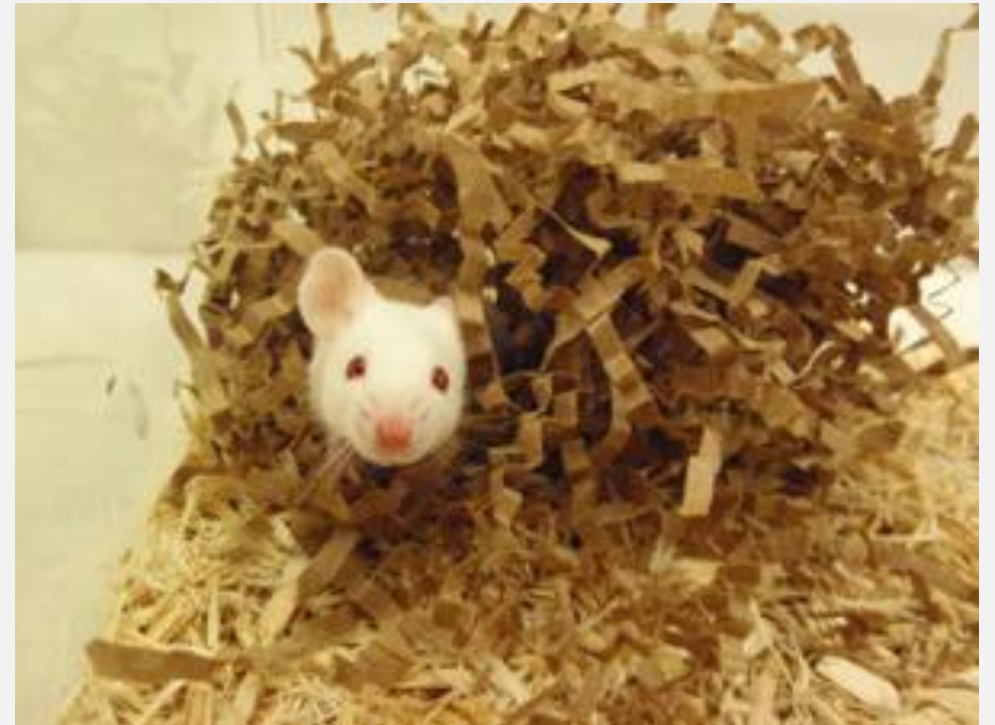
Example: 20 (pups weaned) ÷ 5 (times mated) = 4 pups per month

Factors that influence 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
- ✓ 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

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



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

My Mice are Eating Their Pups!?!?

- ✓ 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

Retire & 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

Dystocia

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

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

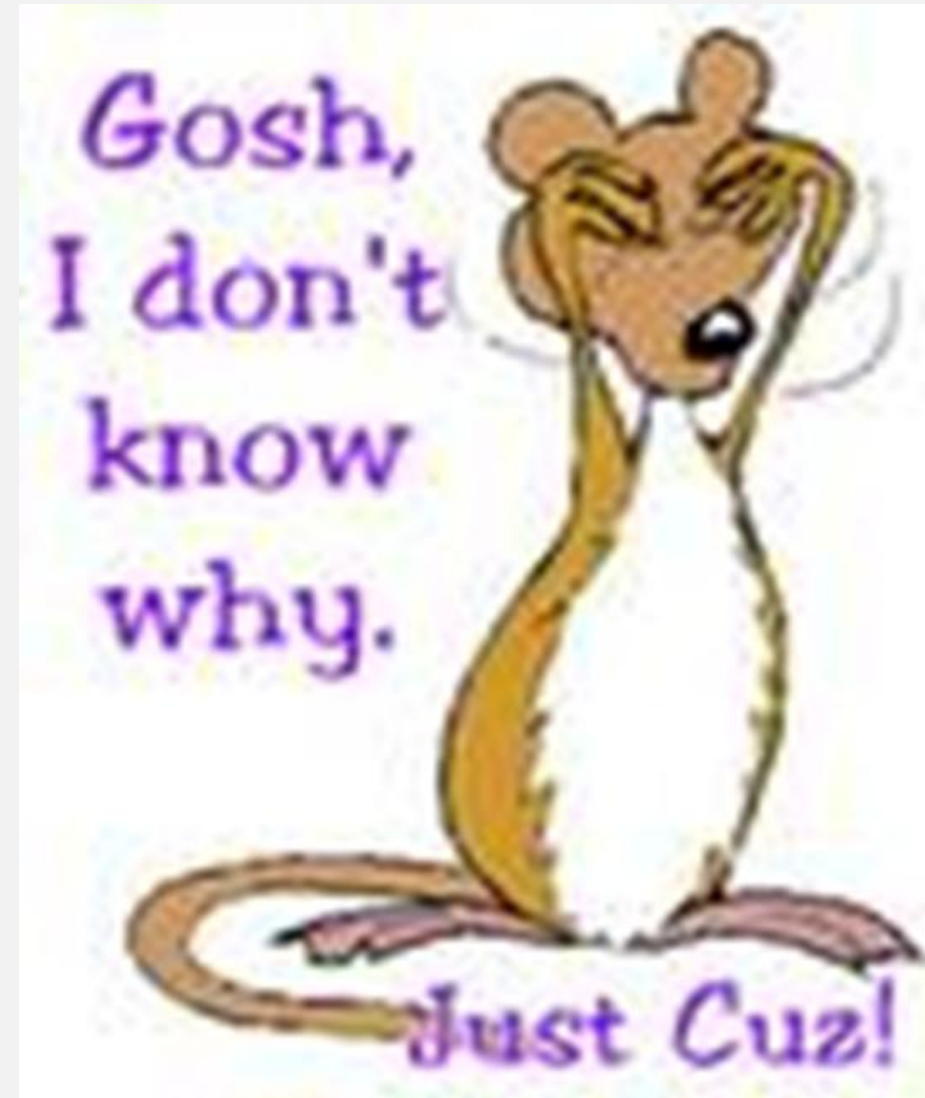
Heroics if needed:

- ✓ Immediately resuscitate any viable pups
- ✓ ****If a valuable female is euthanized or dies, immediately remove ovaries for ovary transplants to maintain gene pool****

Who Lives In a Perfect World?

Sometimes things happen...

- ✓ Moms die
- ✓ Genetic or physical abnormalities prevent moms from caring for litters
- ✓ Some mice are just bad moms!



The Time May Come to Foster a Litter!



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
- ✓ Select moms that have had 2-3
- ✓ successful litters weaned already
- ✓ B6CBAF1 strain make excellent
- ✓ foster moms

When to 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

How to Foster “Keep 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



How to Foster “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

How to Foster “Keep Reminders”

You can never have too many

- ✓ 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



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)
- ✓ 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

Handling Unexpected Results

“Check your Breeding Records”

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

- ✓ 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.

Handling Unexpected Results

“Suspect your Breeders “

- ✓ Eliminate the Affected Cages
- ✓ Replace or Retire old Breeding Pairs
- ✓ Start Over

Dietary Management

Harlan(ENVIGO) Global Rodent Diets



2918 Irradiated

Key Features

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



2919 Irradiated

Key Features

- * 19% Protein
- * 9% Fat

The irradiated version is identical to the standard version, with the exception of packaging

Dietary Management For Weanlings

They may need a softened diet

Dough Diet on
Cage Floor



Moistened Food
Pellets on Cage Floor



H₂O DietGel
76A



Questions



Need Assistance

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“Mouse Breeding Colony Management” Presentation
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