MOUSE BREEDING AND COLONY MANAGEMENT

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Research Manager,
Colony Management Core
THE PLAN

• GENERAL MOUSE REPRODUCTION AND DEVELOPMENT

• COLONY MANAGEMENT:
  ➢ BREEDING SCHEMES/STRATEGIES
  ➢ BREEDING PERFORMANCE FACTORS
  ➢ WEANING, ID AND TISSUE SAMPLING OPTIONS
  ➢ DOCUMENTATION
  ➢ BREEDING TROUBLESHOOTING

• COMMON HEALTH ISSUES

• LONG TERM COLONY MANAGEMENT CONSIDERATIONS

• RESOURCES
### MOUSE BASICS

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<tbody>
<tr>
<td><strong>Lifespan</strong></td>
<td>1.5 to 2.5 years</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>20 to 40 grams</td>
</tr>
<tr>
<td><strong>Length (including tail)</strong></td>
<td>6 to 7 inches (15 to 18 centimeters)</td>
</tr>
<tr>
<td><strong>Mating Age</strong></td>
<td>6-8 weeks of age</td>
</tr>
<tr>
<td><strong>Estrous cycle</strong></td>
<td>4 to 5 days</td>
</tr>
<tr>
<td><strong>Duration of pregnancy</strong></td>
<td>19 to 21 days</td>
</tr>
<tr>
<td><strong>Litter size</strong></td>
<td>2 to 12 young</td>
</tr>
<tr>
<td><strong>Weaning age</strong></td>
<td>21 to 28 days</td>
</tr>
<tr>
<td><strong>Generation Time</strong></td>
<td>12 weeks</td>
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### Estrous Cycle Phases

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
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<tbody>
<tr>
<td>Proestrus (A)</td>
<td>Ovarian follicular development phase</td>
</tr>
<tr>
<td>Estrus (B)</td>
<td>Sexually receptive</td>
</tr>
<tr>
<td></td>
<td>Ovulation</td>
</tr>
<tr>
<td>Metestrus (C)</td>
<td>CL formation, eggs in oviduct</td>
</tr>
<tr>
<td>Diestrus (D)</td>
<td>New wave of follicular development if not pregnant</td>
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</table>

![Images of mouse vulvae showing different estrous cycle phases](image-url)
POSTPARTUM ESTRUS

• FEMALES COME INTO ESTRUS WITHIN 24 HOURS OF PARTURATION

• CAN BE IMPREGNATED IF MALE IS PRESENT

• IDEALLY FIRST LITTER WILL BE READY TO WEAN WHEN SECOND LITTER IS DUE

• TAKE INTO CONSIDERATION WHEN DETERMINING SUITABLE BREEDING SCHEME
MATING COMBINATIONS

<ALWAYS ONLY ONE MALE PER CAGE>

PAIR

TRIO

HAREM

1  ♂  1  ♀

1  ♂  2  ♀

1  ♂  3-4  ♀
### MONOGAMOUS PAIR

**PROS**

- Ease of accurate record keeping
- Continuous breeding, i.e., male and female always together – takes advantage of postpartum estrus
- Parents are known
- Don’t have to separate parents
- Reduces male burnout from excessive breeding
- Exact date of birth for each litter
- Continuous breeding allows for the generation of a max amt of litters per female during her breeding life span

**CONS**

- Higher number of males required
- Higher number of cages required
- If the male is aggressive, he may cannibalize pups and/or injure the female – not common
- If one of the breeding pair dies, it can be difficult to replace
POLYGAMOUS TRIO OR HAREM

**PROS**

- Requires fewer male mice
- Fewer numbers of cages needed
- Can reduce exposure of pups and females to aggressive males
- Allows to produce a large number of litters quickly

**CONS**

- Labor intensive
- Accurate record keeping is more difficult
- IACUC cage density only allows 9 pups total between 2 litters when 3 adults are present
- Most times male must be removed, and females separated because pups total between 2 litters exceeds 11
- Females must be removed from harems when obviously pregnant
- In both situations, unable to take advantage of postpartum estrus and therefore fewer litters per female
- Higher incidence of male burnout
Triple Constraint of a Breeding Project

QUALITY
- Compliance to ideal cohort (e.g. sex, age, etc.)
- Health profile
- Frequency between cohorts
- Proper control animals

SPEED
- Assisted reproductive technology
- Cryopreservation to cohorts
- Peak vs. continuous breeding

COST
- Genotyping
- Animal husbandry
- Microbiological monitoring
HOMOZYGOUS MUTANT (-/-) X HOMOZYGOUS MUTANT (-/-)

- CAN BE USED IF BOTH GENDERS ARE VIABLE AND FERTILE AS HOMOZYGOTES
- OFFSPRING: 100% HOMOZYGOUS; GENOTYPING NOT REQUIRED
- RECOMMEND REGENOTYPING BREEDERS BEFORE PAIRING OR 1ST OFFSPRING LITTER
- MUTANTS SHOULD BE BACKCROSSED TO THE PARENTAL INBRED STRAIN EVERY 10 GENERATIONS TO AVOID GENETIC DRIFT (i.e. RANDOM MUTATIONS EFFECTING PHENOTYPE)
- IF DO NOT BACKCROSS, EVENTUALLY WILL CREATE MUTANTS WITH A DIVERGENT INBRED BACKGROUND (NEW SUBSTRAIN)
- CONTROLS: WILD TYPE CONGENIC STRAIN OR INBRED STRAIN MICE
HETEROZYGOUS MUTANT (+/-) X HOMOZYGOUS MUTANT (-/--) 

• CAN BE USED IF ONLY ONE GENDER IS Viable AND FERTILE AS A HOMOZYGOTE

• OFFSPRING: 50% HOMOZYGOUS; 50% HETEROZYGOUS; GENOTYPING NEEDED

• MUTANTS SHOULD BE BACKCROSSED TO THE PARENTAL INBRED STRAIN EVERY 10 GENERATIONS TO AVOID GENETIC DRIFT (EX: BACKCROSS A MUTANT ON A C57BL/6J BACKGROUND TO THE STANDARD C57BL/6J STRAIN FOR 2 GENERATIONS)

• CONTROLS: +/- CONGENIC STRAIN, NORMAL PHENOTYPE +/- SIBLINGS, OR INBRED STRAIN MICE
HETEROZYGOUS MUTANT (+/-) X HETEROZYGOUS MUTANT (+/-)

- MUST BE USED IF NEITHER GENDER IS VIABLE OR FERTILE AS HOMOZYGOTES
- OFFSPRING: 25% HOMOZYGOUS; 50% HETEROZYGOUS; 25% WILDTYPE; GENOTYPING REQUIRED
- MUTANTS SHOULD BE BACKCROSSED TO THE PARENTAL INBRED STRAIN EVERY 10 GENERATIONS TO AVOID GENETIC DRIFT (EX: BACKCROSS A MUTANT ON A C57BL/6J BACKGROUND TO THE STANDARD C57BL/6J STRAIN FOR 2 GENERATIONS)
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• CONTROLS: +/- OR +/- SIBLINGS OR INBRED STRAIN MICE
TIMED PREGNANCIES

1. HOUSE MALES INDIVIDUALLY FOR 1-2 WEEKS PRIOR TO MATING
   • PICK EXPERIENCED, PROVEN MALES IF POSSIBLE OR USE OLDER (3-4 MONTHS OLD) OVER YOUNGER MALES

2. USE 8-15-WEEK-OLD FEMALES
   • TYPICALLY SEXUALLY MATURE BY 6-8 WEEKS
   • AVOID USING VIRGIN FEMALES OLDER THAN 15 WEEKS, OFTEN MATE LESS RELIABLY

3. GROUP-HOUSE FEMALES AND SYNCHRONIZE THEIR ESTROUS CYCLES PRIOR TO MATING
   • LEE-BOOT AND WHITTEN EFFECT

4. VERIFY ESTROUS STATE OF FEMALES PRIOR TO MATING
   • SHOULD BE IN PROESTRUS OR ESTRUS
   • INSPECT VISUALLY
   • CAN CONFIRM WITH CELL MORPHOLOGY FOLLOWING VAGINAL SWAB

5. ADD ONLY 1-2 FEMALE(S) IN EACH MALE CAGE IN THE AFTERNOON

6. CHECK FOR VAGINAL PLUGS EACH THE NEXT MORNING

7. THE DATE A PLUG IS OBSERVED IS GESTATIONAL DAY 0 TO 0.5

JAX® Mice Pup Appearance by Age

The approximate age of mouse pups can be determined by their physical attributes during the first two weeks of life. Examples of the developmental stages of albino, agouti, and black pups are shown.

Days of Age

To order JAX Mice: 1-800-422-6423 orderquest@jax.org www.jax.org
IACUC REQUIREMENTS FOR BREEDING CARDS

- PI NAME AND PROTOCOL #
- BIRTHDATE OF PUPS IF BREEDING CAGE (REQUIRED WITHIN 3 DAYS OF BIRTH) AND A PINK DCM CARD WITH LITTER DOB AND DOW INSERTED
- PRE-PRINTED CAGE CARD WITH IACUC PROTOCOL NUMBER (DCM RFID CARD)
- NOTE: TEMPORARY CARDS ALLOWED, BUT MUST INCLUDE ALL ABOVE, PLUS DATE THE RFID CARD WAS ORDERED
- CARDS SHOULD ALSO INCLUDE MOUSE STRAIN NAME, GENOTYPE, DOB OF THE BREEDERS, MOUSE IDS, SEXES
BREEDING PERFORMANCE FACTORS

1. BIRTH DEFECTS IN THE PUP
2. OUTBRED VS INBRED STRAINS
3. STRAIN-SPECIFIC BEHAVIORS
4. MUTATIONS AND TRANSGENE EFFECTS
5. TEMPERATURE AND HUMIDITY
6. LIGHT INTENSITY AND LIGHT CYCLE
7. NOISE AND VIBRATION
8. BAROMETRIC PRESSURE
9. ODORS
10. HANDLING
11. NUTRITION
12. FEED
13. FEED PLACEMENT
14. HEALTH

“Don’t play with him, he is Wild Type.”
OPTIMIZING BREEDING PERFORMANCE

• REPLACE BREEDERS BEFORE THEIR REPRODUCTIVE PERFORMANCE DECLINES
• REPLACE NON-PRODUCTIVE BREEDERS
• MATE MICE EARLY
• USE EXPERIENCED MALES
• KEEP METICULOUS AND ACCURATE BREEDING RECORDS
  o INVESTIGATE DEVIATIONS IN BREEDING PERFORMANCE
  o COMPARE YOUR COLONY BREEDING PERFORMANCE TO KNOWN (SUPPLIER, OTHER LABS, ETC.)
  o KEEP ENVIRONMENTAL CONDITIONS SUITABLE AND STABLE
  o PERIODICALLY VERIFY THE GENOTYPES OF BREEDERS/OFFSPRING
• LITTER FOSTERING
• BREEDER DIET VS REGULAR
• MATING NUMEROUS FEMALES SIMULTANEOUSLY
SUCCESSFUL FOSTERING

- CHOOSE FOSTER MOTHER WITH DIFFERENT COAT COLOR AND GREAT MATERNAL BEHAVIOR
- IDEALLY NATURAL PUPS ARE BORN WITHIN 2 DAYS OF THE FOSTER ONES
- KEEP THE LITTER THE SAME (CULL DOWN NATURAL LITTER)
- TAKE THE MOTHER OUT DURING TRANSFER
- RUB SOME URINE, DIRTY BEDDING FROM FOSTER CAGE ON THE PUPS AND THEN MIX THEM WITH BEDDING IN THE NEST
- RETURN THE FEMALE TO THE CAGE AND RETURN THE CAGE TO THE RACK TO AVOID ANY MORE STRESS
- SHE SHOULD START NURSING WITHIN 6 HOURS
• PUPS ARE TO BE WEANED ON DAY 23 IF THEY ARE 10 GRAMS

• MALE AND FEMALE PUPS ARE SEPARATED AT WEANING INTO SEPARATE CAGES WITH NO MORE THAN 5 MICE PER CAGE.

• IF A LITTER CONTAINS A SINGLE ANIMAL OF ONE SEX, THEN PLACE A GEL CUP IN THE CAGE WITH THE PUP.
WEANING

• PLACE A FEW PIECES OF RODENT CHOW ON THE FLOOR OF THE CAGE WITH ALL NEWLY WEANED PUPS

• ADD FOOD TO THE HOPPER AND PROVIDE A CLEAN WATER BOTTLE OR TOGGLE THE LIXIT IF ON AUTOWATER

• PLACE A PINK TOGGLE CARD WITH WEAN DATE +7 DAYS TO MAKE SURE THE LIXIT IS TOGGLED DAILY AND THE MICE ARE CHECKED FOR SIGNS OF DEHYDRATION
RECORD KEEPING METHODS

1. **PAPER/PEN IN FACILITY**: UPDATE ONLINE LATER

2. **LAPTOP/TABLET**
   1. **DATABASES** – MANY SOFTWARE OPTIONS AVAILABLE FOR FREE/PURCHASE
   2. **EXCEL**: CAN BE DIFFICULT WITH MULTIPLE USERS
   3. **GOOGLE SHEETS**: SHAREABLE SECURE EXCEL WITH EDITING CAPABILITIES AND ABILITY TO RESTORE CHANGED INFORMATION
   4. KEEP DATABASE CURRENT
IDENTIFICATION METHODS

- It is important to select the appropriate identification method for your research purposes.
- This should be based upon the age of the animal, the number of characters you wish to include, and the duration of the experiment.
- You should record the identification information on the cage card in the event that clarification of the numbers or characters becomes necessary for any reason.
- Some ID methods can be combined with tissue collection for genotyping to minimize stress to the animal.
IDENTIFICATION METHODS
TEMPORARY

• NON-TOXIC, PERMANENT MARKERS CAN BE USED TO TEMPORARILY MARK THE FUR, TAIL, OR SKIN OF THE ANIMAL

• THIS INK USUALLY LASTS 3-4 DAYS WITHOUT THE NEED TO REMARK
IDENTIFICATION METHODS

EAR NOTCH/PUNCH
IDENTIFICATION METHODS
MOST COMMON EAR NOTCH SYSTEM

- EASY TO READ
- INEXPENSIVE
- CAN USE TISSUE FOR GENOTYPING

HOWEVER:

- SOME STRAINS OF MICE ARE KNOWN FOR EAR MUTILATION
IDENTIFICATION METHODS

EAR TAG

• EASY TO ADMINISTER
• CAN USE LONG NUMBERS

HOWEVER:
• TAGS ARE OFTEN LOST
• MAY CAUSE EAR IRRITATION
• HAVE TO RESTRAINT A MOUSE TO READ THE TAG
IDENTIFICATION METHODS:
MICROCHIP TRANSPONDERS

- Often called “PIT TAGGING”, they are implanted subcutaneously between the scapulae or on the lower back.

- Each microchip is encrypted with a unique, non-replicable number and are read with a portable, hand-held scanner.
IDENTIFICATION METHODS:

EAR RapID TAGS

- PERMANENT ID METHOD
- EASY TO READ
- HYPOALLERGENIC
- SOME SYSTEMS ARE PROGRAMMABLE AND PROVIDE TELEMETRY

HOWEVER:
- EXPENSIVE!!!!
IDENTIFICATION METHODS: TATTOOING

• LOW HEALTH RISK
• CAN BE USED ON ADULTS (TAILS, FOOTPADS, TOES) AND NEONATES (TOES, FOOTPADS)
• VERSATILE

HOWEVER:
• SOMEWHAT DIFFICULT/TIME CONSUMING TO ADMINISTER
• REQUIRES SKILL, EQUIPMENT AND MONEY
• INK FADES OVER TIME
• MUST IMMobilIZE ANIMAL
IDENTIFICATION METHODS: TOEING

- PERMANENT
- EARLY COMBINATION OF ID SYSTEM AND TISSUE SAMPLING FOR GENOTYPING (PD7-10)
- NO MORE THAN 2 TOES PER FOOT OR 4 TOES TOTAL PER ANIMAL

HOWEVER:
- REQUIRES SCIENTIFIC JUSTIFICATION AND IACUC APPROVAL
- MUST PICK UP MICE TO READ
GENOTYPING

- EAR NOTCH/PUNCH
- TOES
- TAIL SNIPS
- BLOOD
- HAIR
- SALIVA
- FECES
GENOTYPING:
TAILS

• MOST COMMONLY USED METHOD IN RODENTS
• A SMALL PORTION OF THE DISTAL TAIL IS AMPUTATED
• CAN BE USED WITH PUP 10 – 15 DAYS OLD OR OLDER
• REMOVE 2MM OF THE DISTAL TAIL
• PLACE THE MOUSE IN A RESTRAINER OR ANESTHETIZE
• USE A SCALPEL OR SCISSORS TO REMOVE THE TISSUE
• THE INSTRUMENTS NEED TO BE STERILE AT THE BEGINNING OF THE PROCEDURE AND SANITIZED BETWEEN ANIMALS
• YOU MUST ASSURE THAT ADEQUATE HEMOSTASIS HAS BEEN ACHIEVED BEFORE RETURNING THE ANIMAL TO ITS CAGE
• SURGICAL GLUE, SILVER NITRATE, OR DIRECT PRESSURE WITH A STERILE PAD CAN BE USED FOR THIS PURPOSE
BREEDING TROUBLESHOOTING

• IF LITTER IS CANNIBALIZED, CONSIDER
  • IS IT MOM’S FIRST LITTER?
  • IS THE MALE AGGRESSIVE?
  • IS THERE A SECOND LITTER THERE?
  • WAS THE LITTER HANDLED TOO EARLY

• WHERE THE MICE RECENTLY MOVED OR CHANGED CAREGIVERS

• CHANGES IN SMELL, TEMPERATURE, NOISE, LOCATION ON THE RACK

• BREEDERS TOO OLD/YOUNG/UNHEALTHY
  • OLDER MICE HAVE SMALLER, LESS FREQUENT LITTERS
  • CAN HAVE PROBLEMS DELIVERING

• CONSIDER DIET CHANGE TO A HIGHER FAT BREEDER CHOW

• GENETIC COMPOSITION OF YOUR BREEDERS MAY PRODUCE EMBRYONIC LETHAL MUTATION OFFSPRING
UNHEALTHY MOUSE

• SIGNS THAT YOUR MOUSE IS SICK:
  • SCRUFFY COAT
  • HUNCHED, SUNKEN AT HIPS
  • WEIGHT LOSS, LOW ACTIVITY
  • SKIN LESIONS
  • LABORED BREATHING
  • EYE OR NASAL DISCHARGE
  • ABNORMAL BEHAVIOR
  • HYPOTHERMIA/LETHARGY
UNHEALTHY MOUSE = BAD FOR BREEDING

- **EXAMPLE: MALOCCLUSION**
  - TEETH OVERGROW CHRONICALLY, HAVE TO TRIM WEEKLY
  - GENETIC PREDISPOSITION
  - DO NOT USE AS BREEDERS

- **COMMON IN PREGNANT DAMS:**
  - PROLAPSED UTERUS/VAGINAL TISSUE
  - DYSTOCIA (PROLONGED/DIFFICULT LABOR)
  - DEHYDRATION
BREEDING COLONY LONG TERM CONSIDERATIONS

- Maintain founder stocks or refresh breeders by purchasing every 10 generations.
- Backcross your breeders 2 generations (fixing for sex chromosomes) or start with brand new purchased.
- Check for genetic quality control.
- Consider having strains tested by an outside source for "in/purity".
- Dartmouse/Jackson Labs/Charles River.
- Consider cryopreservation.
- Freeze embryos/sperm of non actively used strains to preserve genetic integrity.
- Saves $$$ long term (effort, housing costs, genetic testing, etc.).
- Provides a way to eliminate pathogens.
- Contact MMRR@UNC for more information.
RESOURCES

• FUNDAMENTALS OF BREEDING VIDEO:
  • HTTPS://WWW.JOVE.COM/VIDEO/10293

• JACKSON LABORATORIES:
  • HTTPS://WWW.JAX.ORG/NEWS-AND-INSIGHTS/JAX-BLOG/2014/DECEMBER/FIVE-REASONS-WHY-YOUR-MICE-ARE-NOT-BREEDING
  • HTTPS://WWW.JAX.ORG/-/-MEDIA/JAXWEB/FILES/JAX-MICE-AND-SERVICES/COLONYSIZEWKSHT.PDF?LA=EN&#HASH=8C1CB11B342A9E8177B31DD2D7A7BFC6414628AC
  • HTTPS://WWW.RESEARCH.uci.edu/FORMS/DOCS/IACUC/JAX-BREEDING-STRATEGIES.PDF
COLONY MANAGEMENT CORE AT UNC (CMC)

• FOCUS ON ESTABLISHING, MAINTAINING, AND CONTINUOUSLY MANAGING RESEARCH COLONIES FOR INVESTIGATOR USE

• OUR SERVICES ALLOW FOR RESEARCH PERSONNEL TO FOCUS ON SCIENCE
  • REDUCE EXCESSIVE MOUSE PRODUCTION
  • ASSIST IN ESTABLISHING NEW STRAIN LINES
  • MINIMIZE BREEDING DISRUPTIONS AND STRESS TO THE ANIMALS

• SERVICES ARE CUSTOM SELECTED TO SUIT EACH LABORATORIES' NEED AND BUDGET
  • AID WITH MAINTENANCE AND PRODUCTION COLONIES
  • ABLE TO HANDLE LARGE COLONIES WITH MULTIPLE STRAINS/GENOTYPES

• SKILLED, DEDICATED BREEDING SPECIALISTS PERFORM DAILY TASKS
  • YEARS OF EXPERIENCE WITH TRANSGENIC COLONIES
  • HANDS ON AT UNC

• SECURE, SHAREABLE MOUSE DATABASE UPDATED IN REAL TIME
  • CURRENTLY USE GOOGLE SHEETS (CAN BE VIEWED/EDITED BY ALL ADDED INDIVIDUALS)
  • PILOTING A COLONY MANAGEMENT DATABASE WITH TRANSNETYX
  • UPDATED BETWEEN 2 TO 5 TIMES A WEEK DEPENDING ON THE SIZE AND NEEDS OF COLONY

• FOR MORE INFORMATION: CONTACT NATALLIA RIDDICK, PhD (CORE MANAGER) TO DISCUSS INTEREST

https://research.unc.edu/cmc/
THANK YOU!

QUESTIONS?