Reproductive Biology of Breeding Mice

Brian W. Soper, PhD
Technical Information Scientist
The Jackson Laboratory’s Mission

Performing Research: investigating genetics and biology of human disease

Providing Resources: JAX® Mice & Services, bioinformatics data, technical publications and more

Educating Scientists: world-class courses, internships and other programs

www.jax.org/courses
JAX® Mice
The *Gold Standard* for Biomedical Research

- NIH funded resource
- 6,000 strains and growing
  - 2.7 million mice shipped annually
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, DBA/2J) support development/collection of specialty strains and other valuable community research resources
The Jackson Laboratory

World renowned non-profit genetics research institute and international training center

Bar Harbor, Maine

Leading provider of genetically defined mice and services including pre-clinical research

Sacramento, Calif.
The Jackson Laboratory for Genomic Medicine
Online Resources to Expedite Research

- JAX® Mice Database
  www.jax.org/jaxmice

- Mouse Genome Informatics
  www.informatics.jax.org

- Mouse Phenome Database
  www.jax.org/phenome

- And many more unique resources

www.jax.org/jaxmice/support/techsupport-index
BALB/cJ Reproduction Data in MPD

http://phenome.jax.org/db/q?rtn=strains/details&strainid=5
BALB/cJ – Assisted Reproduction Data
Comparatively poor oocyte yield following superovulation
Overview

Reproductive Biology of the Mouse

• Reproductive characteristics
• Determining gender
• Male & female anatomy and gamete production
• Reproductive states in female mice
• Estrous cycle
  – Stage detection
  – Interruption
• Troubleshooting non-productive animals
Reproductive Characteristics

- Sexual maturity: 5 to 8 weeks
- Estrous cycle: ~4 days; spontaneous
- No. eggs ovulated: 6-16 (variable by strain)
- Gestation: 18.5 – 21 days
- Litter size: 2 to 12+ pups
- Productive breeding life: ~7-8 months
Sex Determination: Adult Mice

MALE

FEMALE
Sex Determination: Weanlings

MALE

FEMALE
Sex Determination: Newborns

MALE

FEMALE
Male Anatomy

Seminal vesicle
Coagulating gland
Ductus deferens
Caput epididymis
Right testis
Cauda epididymis
Gubernaculum

Left ureter
Prostate
Cut end of pelvic girdle
Preputial gland
Penis

(Bulbourethral gland)

Cook MJ. 1965. The Anatomy of the Laboratory Mouse
http://www.informatics.jax.org/cookbook/
Male Anatomy

- Vas Deferens
- Seminal Vesicles
- Prostate
- Coagulating Gland
- Bladder
- Epididymis
- Testes
- Penis

Sperm Production

- Diploid spermatogonial stem cells – lifetime supply
- 12 stages of spermatogenesis in the seminiferous tubules
  - 35 days
- Transport to epididymis for storage and maturation (motility)
- For IVF, collect sperm from the epididymis

http://www.biostr.washington.edu/Art/Koehler/koehler
Female Anatomy

Bursa → Ovary

Oviduct

Cook MJ. 1965. The Anatomy of the Laboratory Mouse
http://www.informatics.jax.org/cookbook/
Oocyte (Egg) Production

- Females born with finite number of oocytes
- At 6 weeks, each ovary has 10,000 oocytes
- 6-16 oocytes ovulate over 2-3 hours every 4-5 days in mice
Reproductive States of Adult Female Mice

- Cycling
- Pregnant
- Pseudopregnant
  - e.g. seasonal non-cycling
- Reproductively senescent

**Estrus & Ovulation under Neuroendocrine Control**

- Hypothalamus (GnRH)
- Pituitary (FSH & LH)
- Ovary (E & P)
- Uterus
- (E & P) (Inhibin)
- (Pg)
The Estrous Cycle

Stages (~4 days per cycle)

- Proestrus (13 hrs)
- Estrus- ovulation (15 hrs)
- Metestrus (13 hrs)
- Diestrus (56 hrs)

Cycle Interruption

- Mating
- Pheromones
- Environment
- Exogenous hormones

Select proestrus/estrus females:

- Timed matings
- Pseudopregnant females
  - vasectomized males required

Detecting Estrous Cycle Stage

Hormonal Changes During Estrous

- Estrogen
- LH
- FSH

Hormone concentration over time:
- Proestrus
- Estrus
- Metestrus
- Diestrus
- Proestrus
Estrous Cycle Interruption: Mating

- Best observed early morning
- Produced by seminal vesicle and coagulating gland
- Prevents mating with other males
- Plug does not guarantee pregnancy
- Mating stimulation (not plug) induces pseudopregnancy
Hormonal Changes During Pregnancy

Hormone concentration

- Progesterone
- Estrogen
- LH
- FSH

Mating

Pregnancy/Pseudopregnancy

time
Estrous Cycle Interruption: Pheromones

Lee-Boot Effect
- S. Van der Lee and L.M. Boot, 1955
- Group housed females isolated from male mice tend to cease cycling
- Females enter anestrus

Whitten Effect
- Wesley K. Whitten, 1956
- Pheromones in male urine induce estrus in females
- used to synchronize estrus in females
Estrous Cycle Interruption: Pheromones

Bruce Effect - 1959
• Exposure of a pregnant female to an unknown male results in pre- or post-implantation failure

Vandenergh Effect - 1969
• Adult males accelerate puberty in pre-pubertal females

Hilda Margaret Bruce
JG Vandenergh
Estrous Cycle Interruption:
Superovulation with Exogenous Hormones

• Pregnant Mare Serum Gonadotropin (PMSG)
  – Acts like follicle stimulating hormone (FSH)
  – Induces follicular development
  – After 48 hours, follicles begin to degrade

• Human Chorionic Gonadotropin (hCG)
  – Acts like luteinizing hormone (LH)
  – Induces ovulation ~12 hrs post hCG

• Considerations:
  – Mating occurs in proestrus/early estrus ~mid-night (dark cycle)
  – Superovulation success is strain specific
  – Size/age
## Reproductive Characteristics of Inbreds

<table>
<thead>
<tr>
<th>Strain</th>
<th>Productive matings</th>
<th>Weeks at first mating</th>
<th>Litter size</th>
<th>No. of litters</th>
<th>Relative fecundity</th>
<th>Response to superovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/SvJ</td>
<td>75%</td>
<td>7.9</td>
<td>5.9</td>
<td>4.1</td>
<td>18.1</td>
<td>High</td>
</tr>
<tr>
<td>A/J</td>
<td>65%</td>
<td>7.6</td>
<td>6.3</td>
<td>2.9</td>
<td>11.9</td>
<td>Low</td>
</tr>
<tr>
<td>AKR/J</td>
<td>84%</td>
<td>6.6</td>
<td>6.1</td>
<td>2.2</td>
<td>11.3</td>
<td>-</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>47%</td>
<td>8.0</td>
<td>5.2</td>
<td>3.8</td>
<td>9.3</td>
<td>Low</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>86%</td>
<td>6.7</td>
<td>5.7</td>
<td>2.9</td>
<td>14.2</td>
<td>Low</td>
</tr>
<tr>
<td>C3H/HeOuJ</td>
<td>99%</td>
<td>5.9</td>
<td>6.4</td>
<td>3.7</td>
<td>23.4</td>
<td>-</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>84%</td>
<td>6.8</td>
<td>7.0</td>
<td>4.0</td>
<td>23.5</td>
<td>High</td>
</tr>
<tr>
<td>C57 BL/10SnJ</td>
<td>67%</td>
<td>7.7</td>
<td>6.3</td>
<td>2.8</td>
<td>11.8</td>
<td>-</td>
</tr>
<tr>
<td>CBA/CaJ</td>
<td>96%</td>
<td>6.4</td>
<td>6.9</td>
<td>2.7</td>
<td>17.9</td>
<td>High</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>75%</td>
<td>7.4</td>
<td>5.4</td>
<td>3.9</td>
<td>15.8</td>
<td>Low</td>
</tr>
<tr>
<td>FVB/N</td>
<td>&gt;90%</td>
<td>-</td>
<td>9.5</td>
<td>4.8</td>
<td>41.0</td>
<td>Moderate</td>
</tr>
<tr>
<td>SJL/J</td>
<td>72%</td>
<td>7.4</td>
<td>6.0</td>
<td>3.1</td>
<td>13.4</td>
<td>High</td>
</tr>
</tbody>
</table>

Reproductive Success: Environment Effects

- Nutrition
- Light cycle and intensity (14hr light/10hr dark)
- Stress: noise, vibrations, odors, over-handling
  - Breeding cessation
  - Resorption of fetuses
  - Cannibalism of litters
- Health status
- Seasonal effects

www.jaxmice.jax.org/support/husbandry/room-conditions
Seasonal Variation in Inbred Mice
Mating Options

• Pair: one female x one male

• Trio: two females x one male (same cage)
  – “Aunting” phenomenon

• Harem: single male, more than two females
  – NOT recommended

• Male rotation: two females x male (week 1), same male, two new females (week 2)
  – Single mutant male, need many offspring
  – Male has a very short lifespan (neuro. mutants)
Breeding Tips for Low Producing Strains

- Quiet place
- Ensure adequate darkness
- Minimal handling
- Use clean forceps or gloves
- Change dietary fat content
- Add enrichment
- Leave mating pairs together

www.jaxmice.jax.org/support/husbandry/index
Troubleshooting Non-Reproductive Mice

My mice

- Don’t get pregnant
- Get pregnant, but never give birth
- Give birth, but pups die
- Not enough pups
Troubleshooting

• Has anything in the room changed?
• Do the animals appear healthy?
• Are nearby strains having problems?
• What is the breeding history?
• Is this strain prone to problems?
Strategies—Non-Productive Males

- Mate to new female
- Check for copulation plug
- Surgically evaluate reproductive tract
- Interventions:
  - Artificial Insemination
  - *In vitro* fertilization (IVF)
  - Intracytoplasmic sperm injection (ICSI)
Strategies—Non-Productive Females

- Mate to new male
- Check for copulation plug
- Check pups for milk spots
- Surgically evaluate reproductive tract
- Interventions:
  - Ovary transplant
  - Low dose gonadotropins
  - *In vitro* fertilization (IVF)
  - Fostering
How to Foster a Litter

- Select foster mother
  - different coat color
  - has successfully weaned a litter (ideal)
  - has a near age matched litter (ideal)
- Remove natural litter and reduce in size
- Combine foster and natural pups
- Gently mingle pups with soiled shavings from foster cage
- Place all pups in foster cage
- Do not disturb
- Pups gathered into the nest is a good sign

www.jaxmice.jax.org/support/husbandry/fostering-litters
Reducing Costs

• Size colony for your needs
• Use both genders or age range of mice
• Mate early, rotate breeders regularly & replace nonproductive breeders ASAP
• Try JAX Colony Management System www.jax.org/jcms
• Considering purchasing cohorts of mice
• Cryopreserve unique and low-use strains
Cryopreservation

Be assured that your mice are protected... cryopreserve them!

- each strain takes 2-3 years and more than $100,000 to create

Can you afford not to preserve your strains?
The Jackson Laboratory
Genetic Stability Program

Frozen embryos used to refresh foundation stock every five generations

Foundation Stock

Expansion & Distribution

25 yrs Frozen Stock

US patents 7592501, 8110721

www.jaxmice.jax.org/genetichealth/stability
Strain Rescue

• For small colonies threatened by old age, breeding cessation, or poor health
• Advanced techniques used to attempt to rescue your strain, including
  – Superovulation, sperm collection & \textit{in vitro} fertilization
  – Ovarian transplantation
  – Hysterectomy derivation
• Successfully rescued \textasciitilde100 strains from extinction
• Call us before it is too late!

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