Reproductive Biology of Breeding Mice

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Technical Information Scientist

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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

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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

Ask for the J

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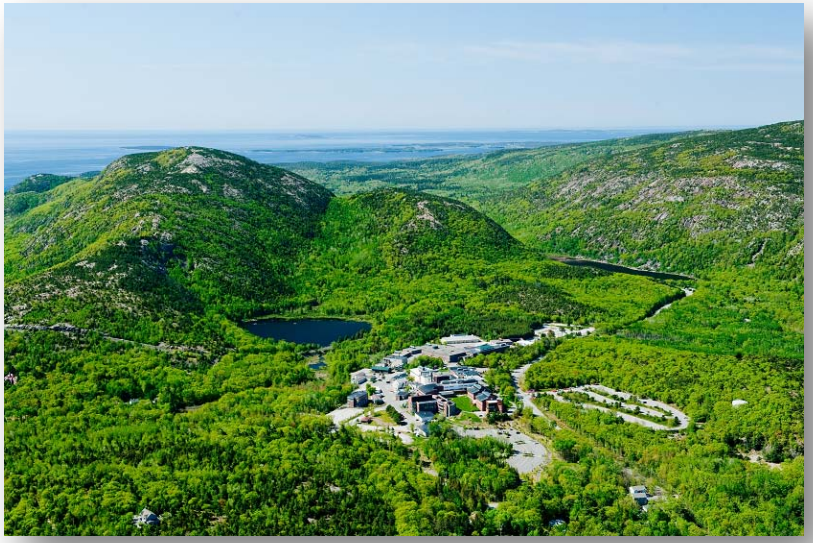

Bar Harbor, Maine

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

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

Sacramento, Calif.

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Farmington, CT

The Jackson Laboratory for Genomic Medicine

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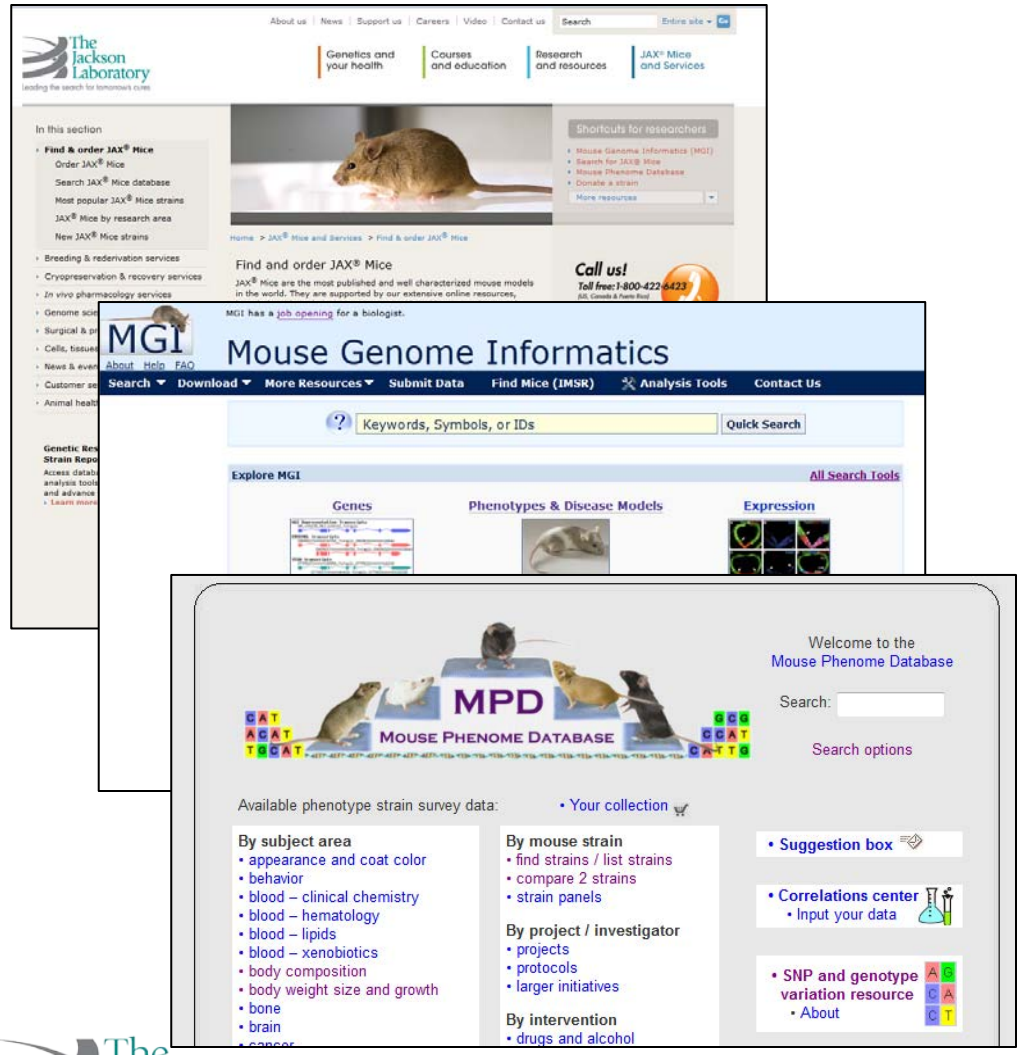
Online Resources to Expedite Research



- **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*

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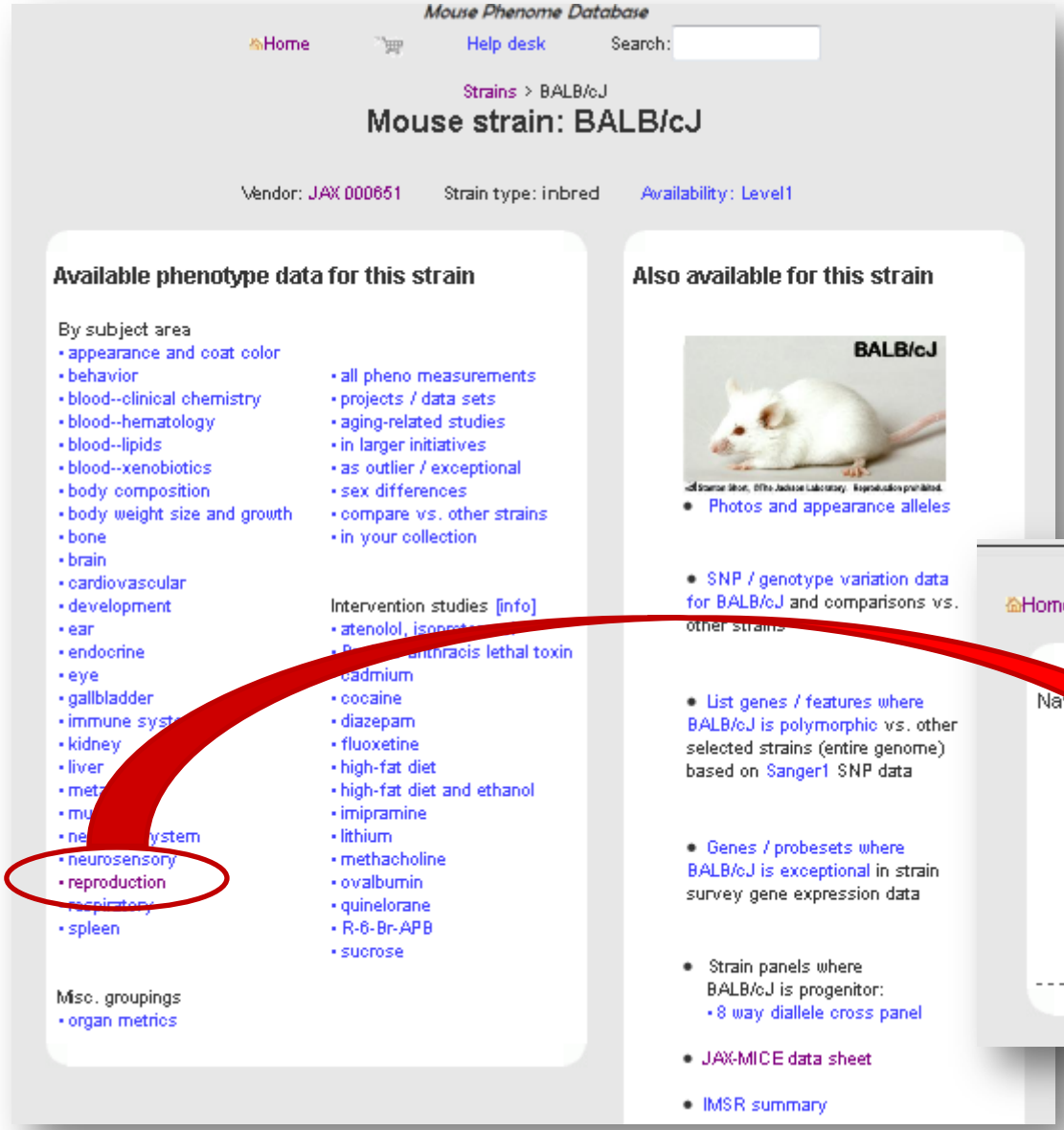
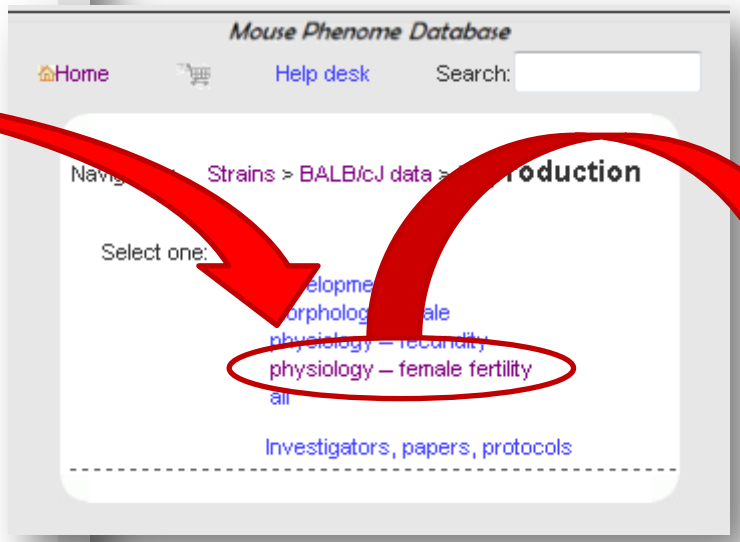
www.jax.org/jaxmice/support/techsupport-index

BALB/cJ Reproduction Data in MPD

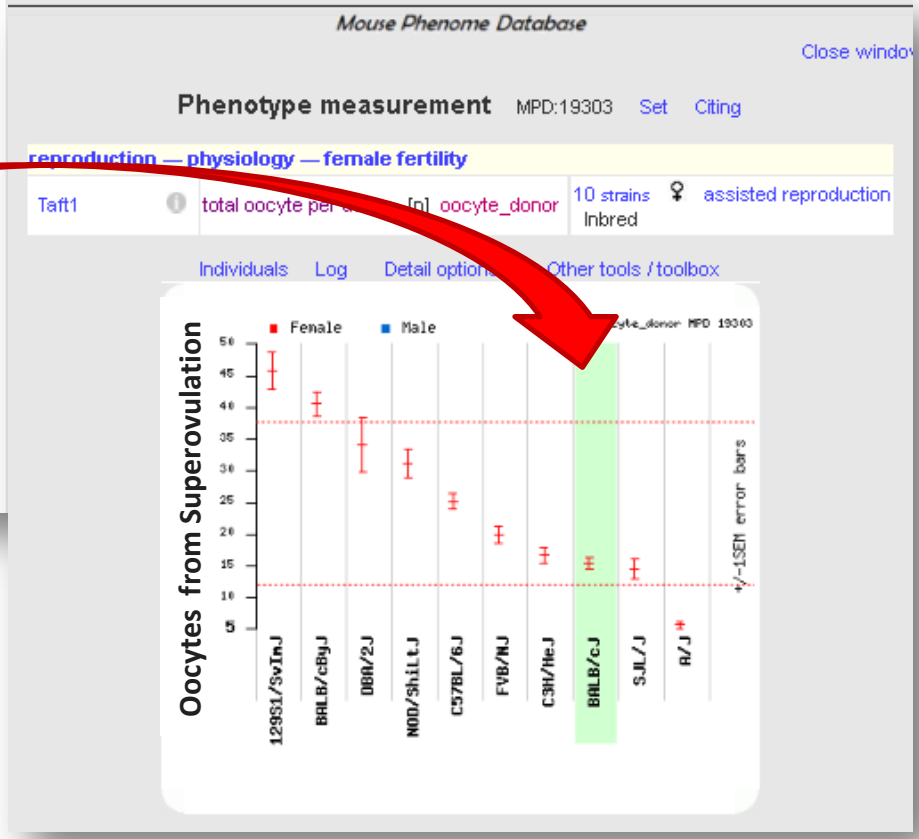




http://phenome.jax.org/db/q?rtn=strains/details&strainid=5

BALB/cJ – Assisted Reproduction Data

Comparatively poor oocyte yield following superovulation



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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

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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

Silver LM. 1995. Mouse Genetics: Concepts and Applications, Oxford University Press. Available online at www.informatics.jax.org/silver

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Sex Determination: Adult Mice

MALE FEMALE

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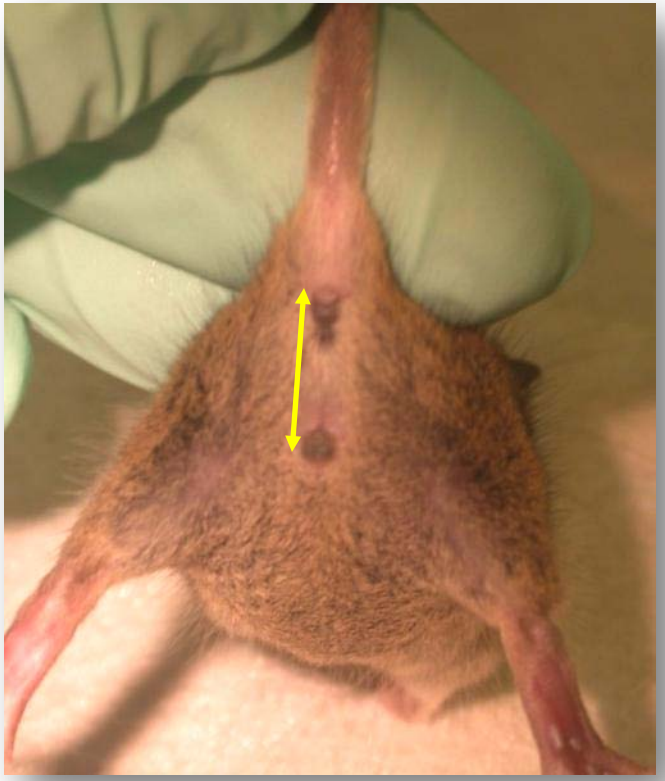
Sex Determination: Weanlings



MALE

FEMALE

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Sex Determination: Newborns

MALE

FEMALE

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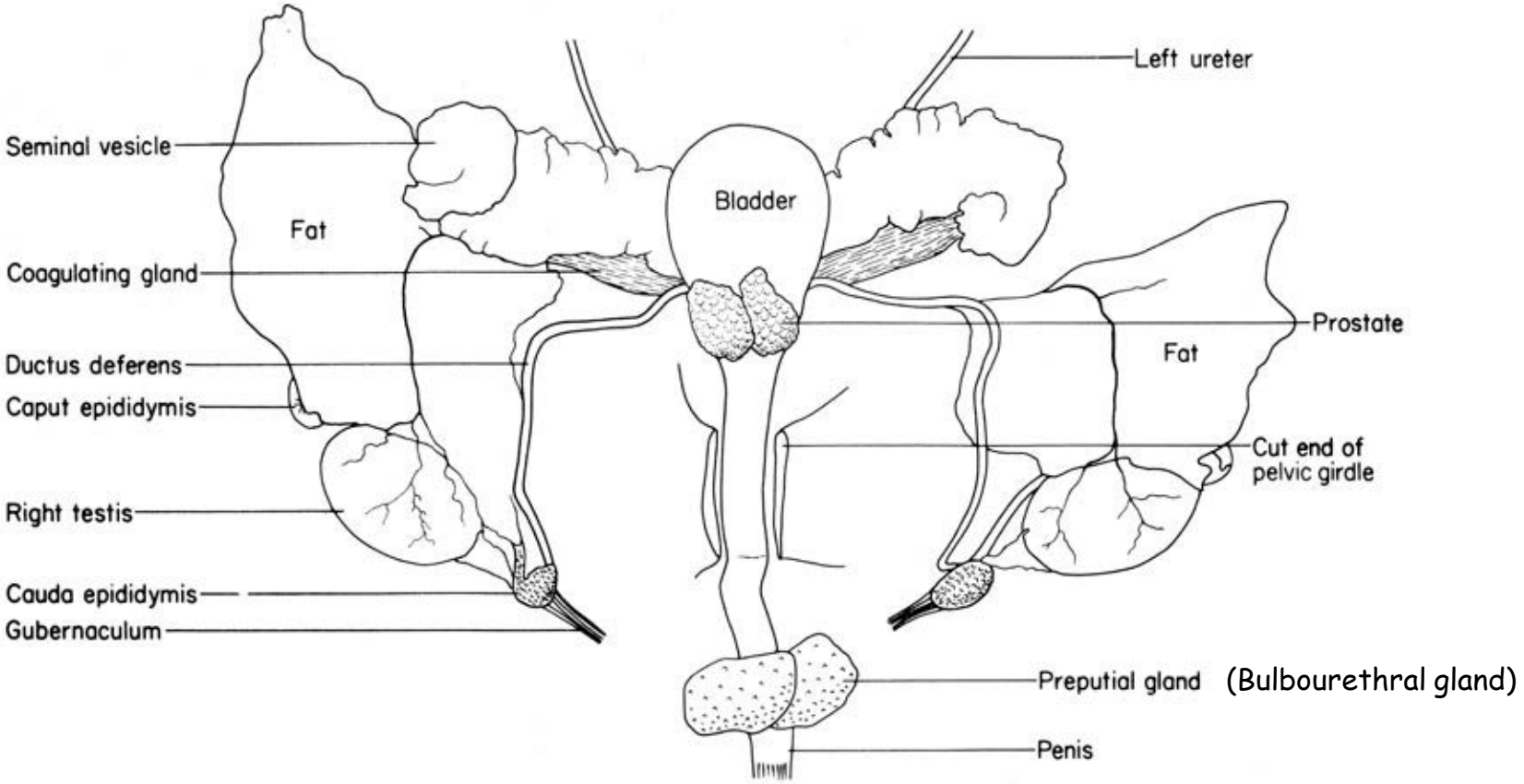
Male Anatomy



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

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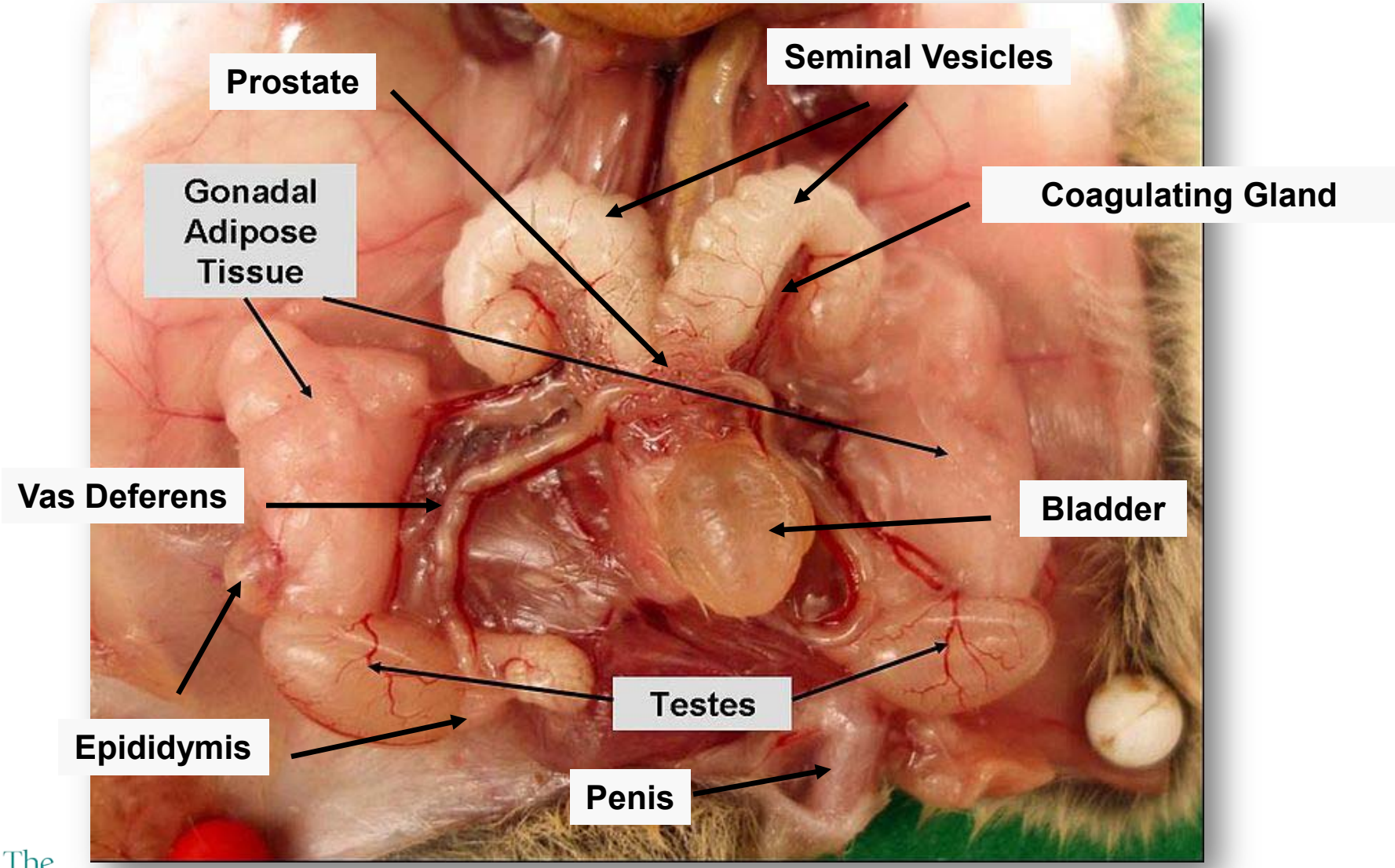
Male Anatomy



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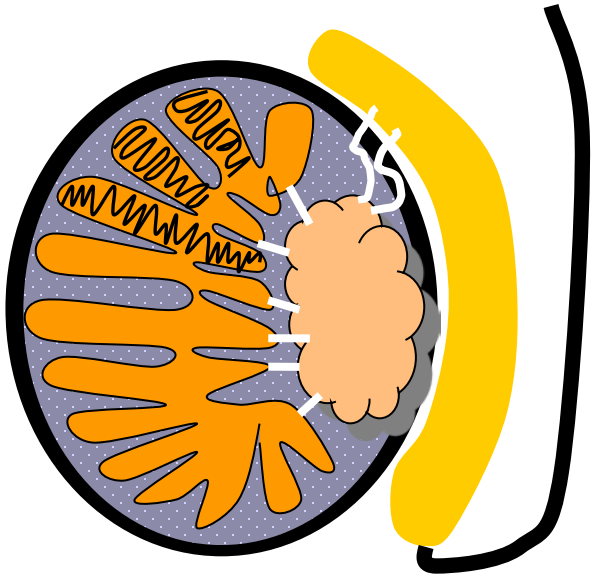
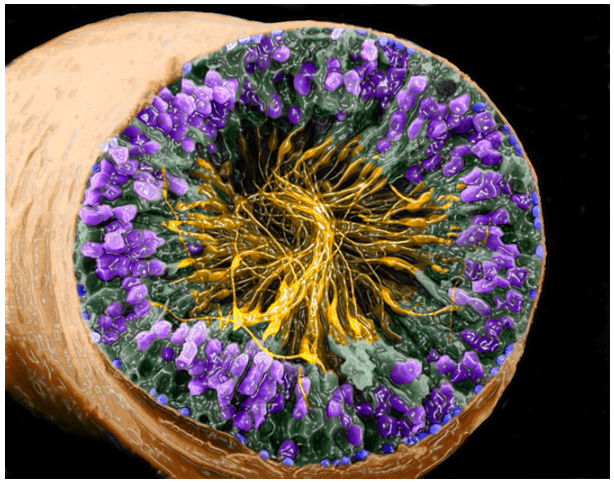
http://en.wikipedia.org/wiki/File:Male_gonadal.jpg

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





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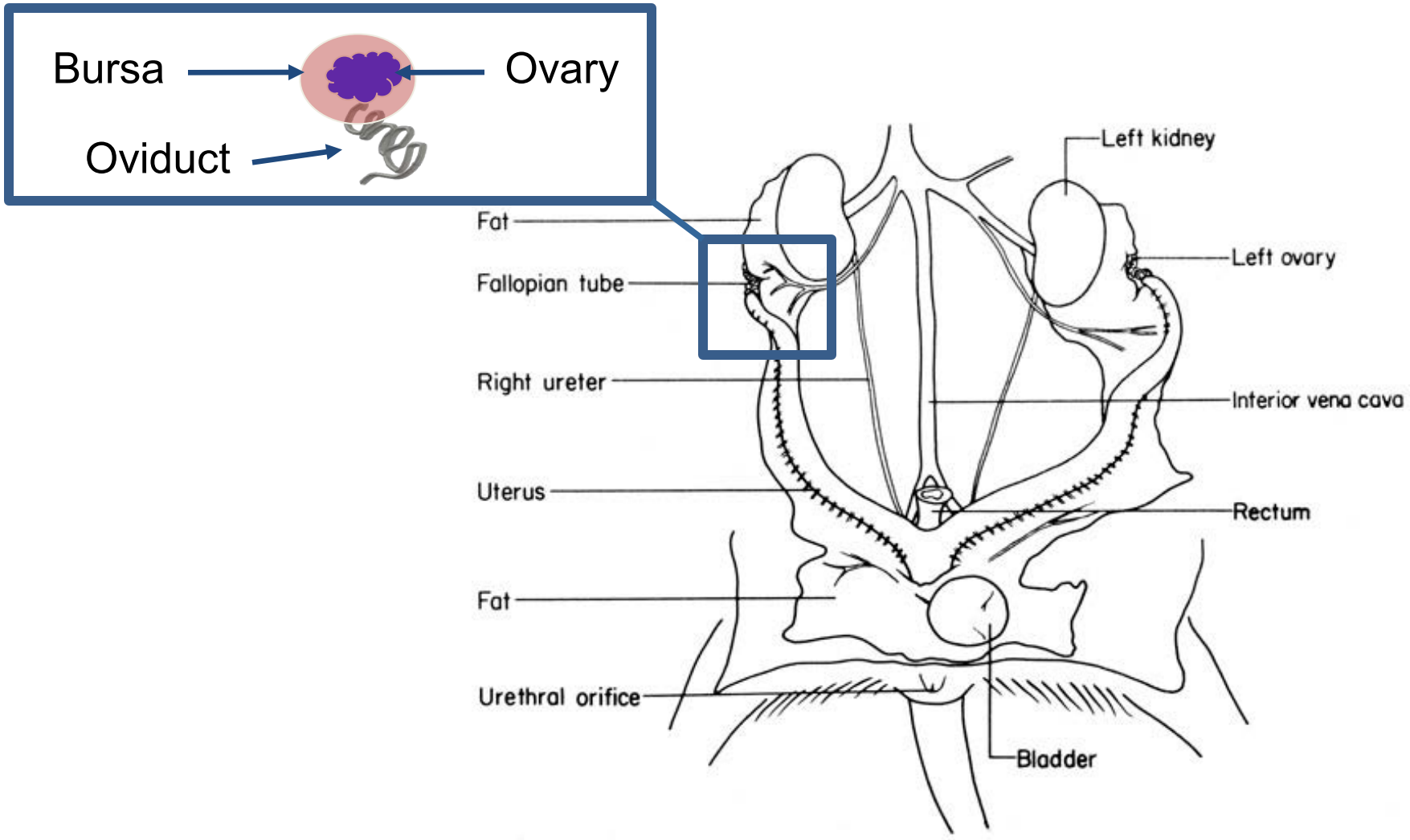
http://www.biostr.washington.edu/Art/Koehler/koehler

Female Anatomy



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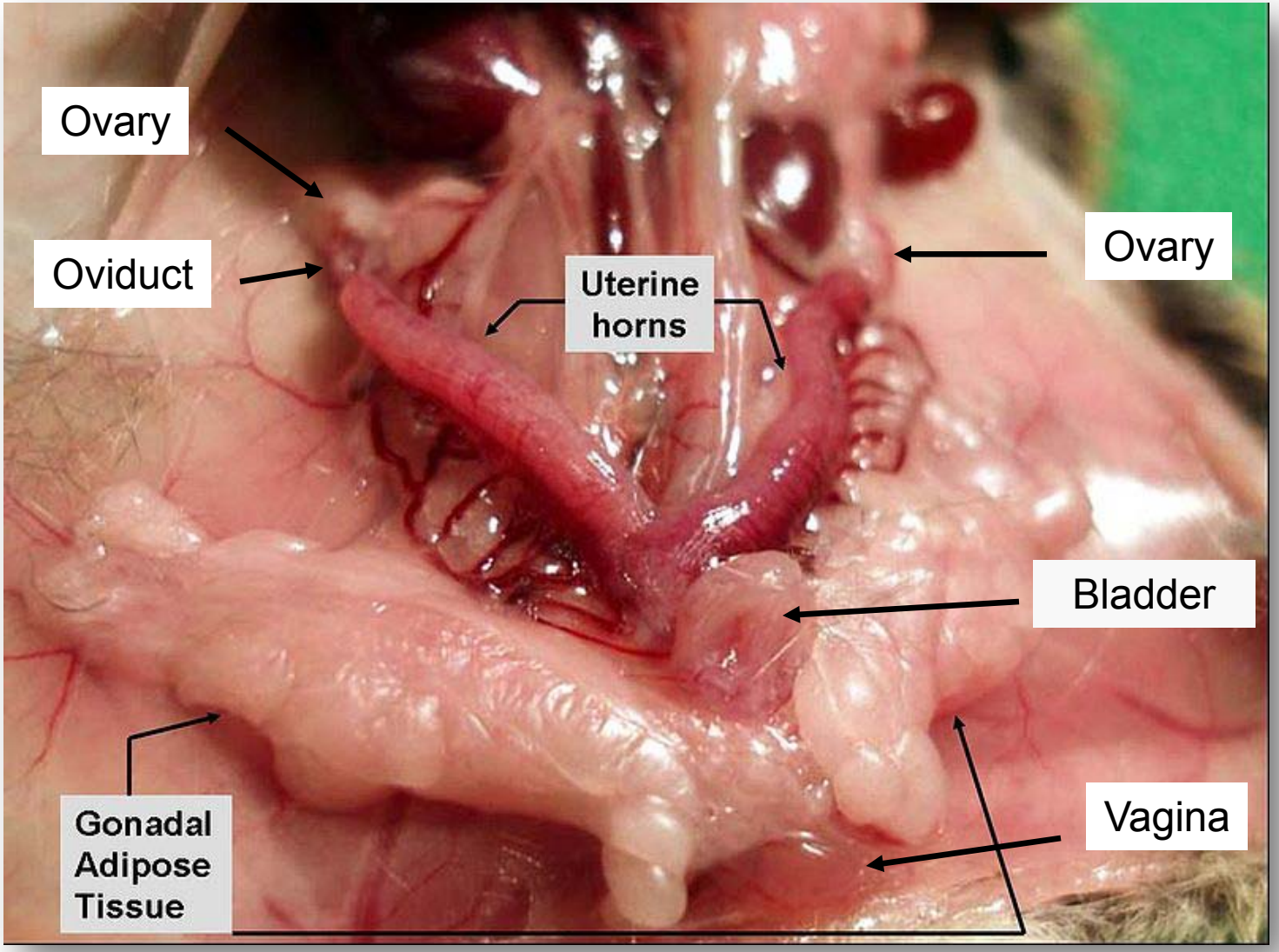
Female Anatomy



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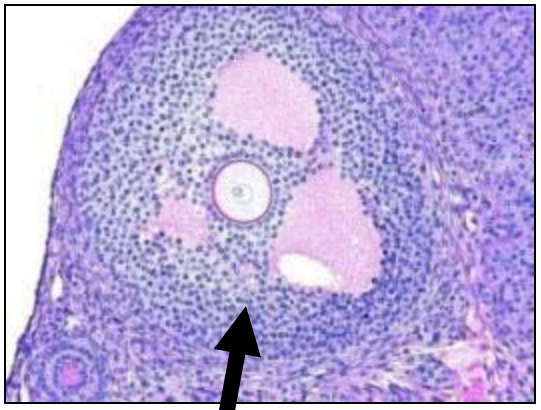
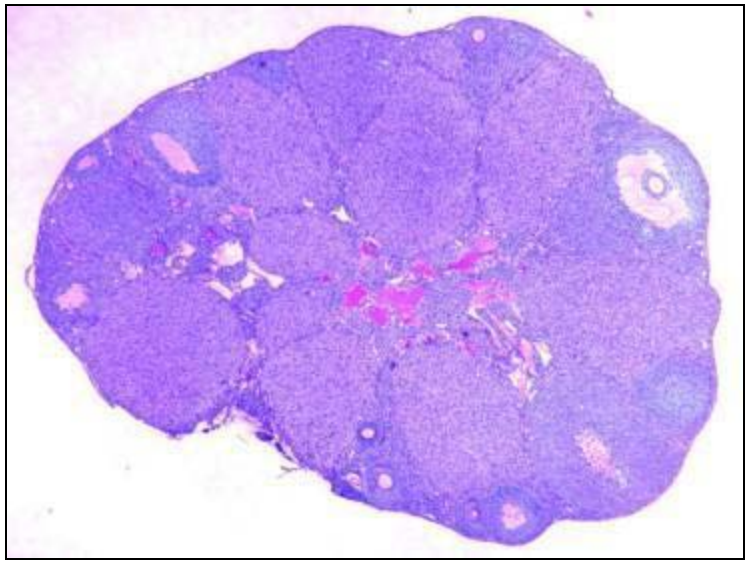
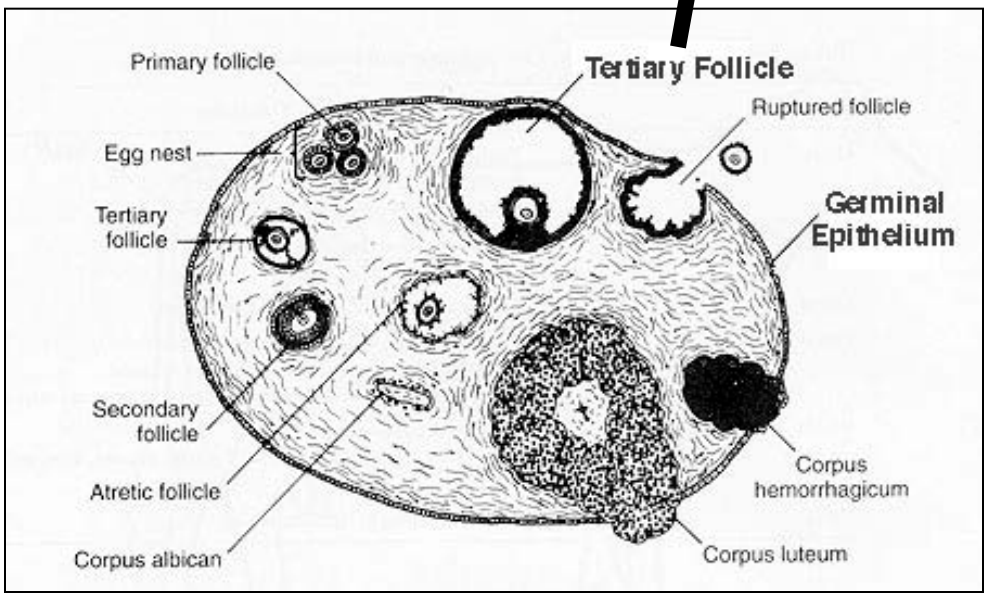
http://en.wikipedia.org/wiki/File:Female_gonadal.jpg

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







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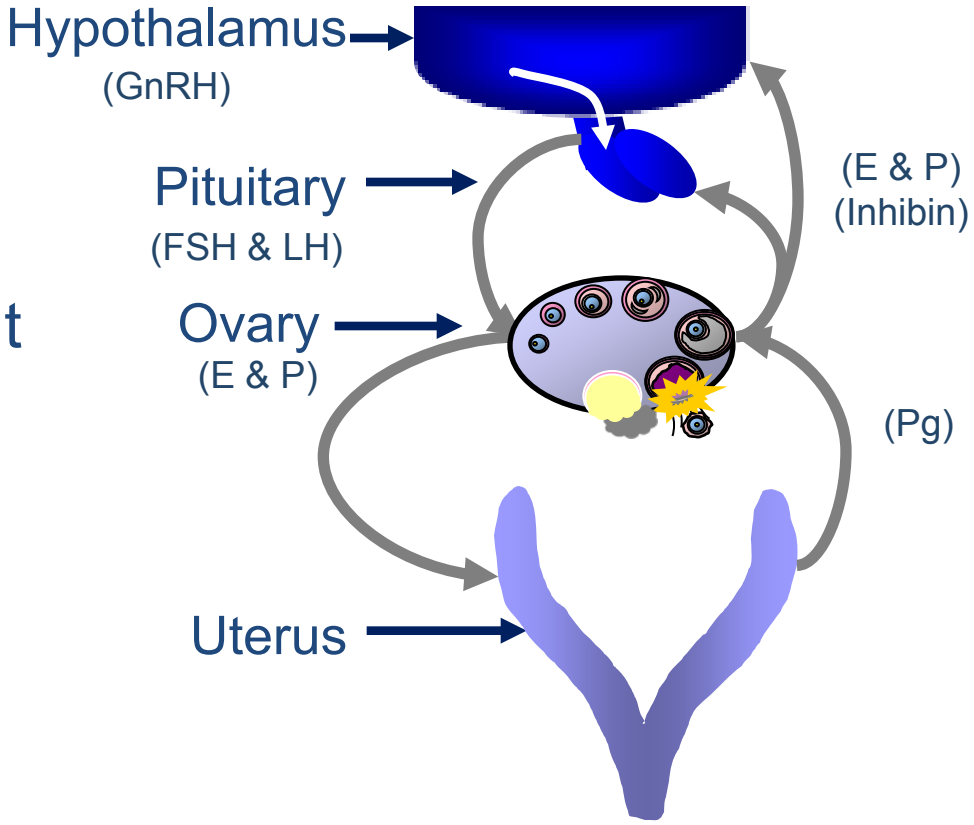
Reproductive States of Adult Female Mice

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

Estrus & Ovulation under
Neuroendocrine Control



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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

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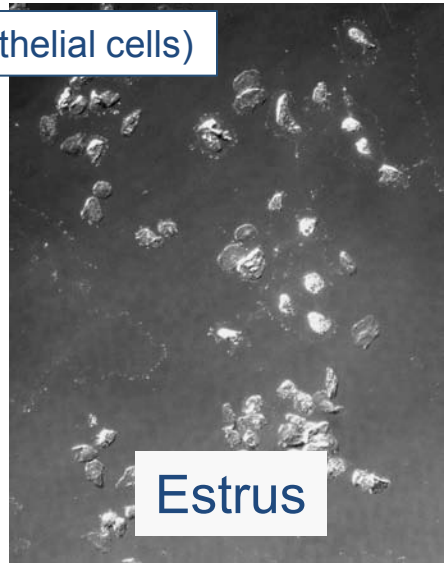
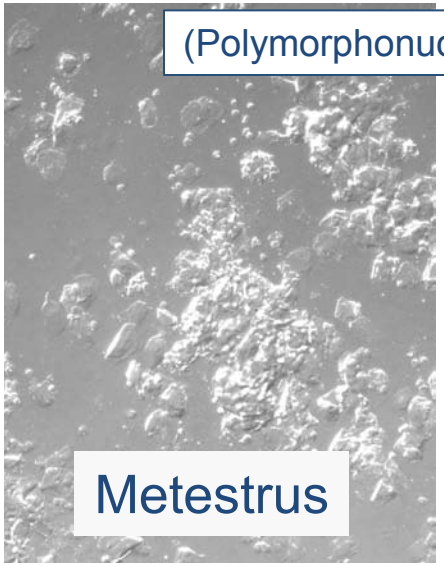
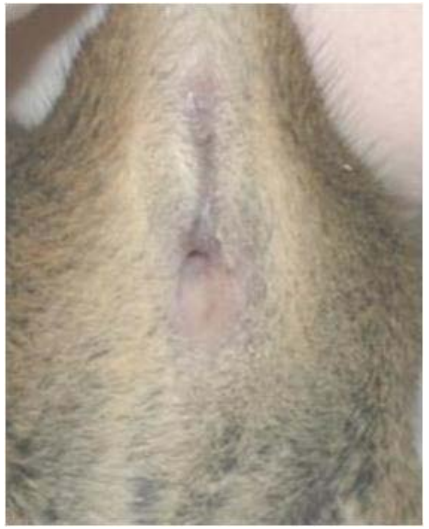
Grasso P *et al*. 1998. *Biol Reprod* 58(3):821-5. PMID: 9510972

Detecting Estrous Cycle Stage

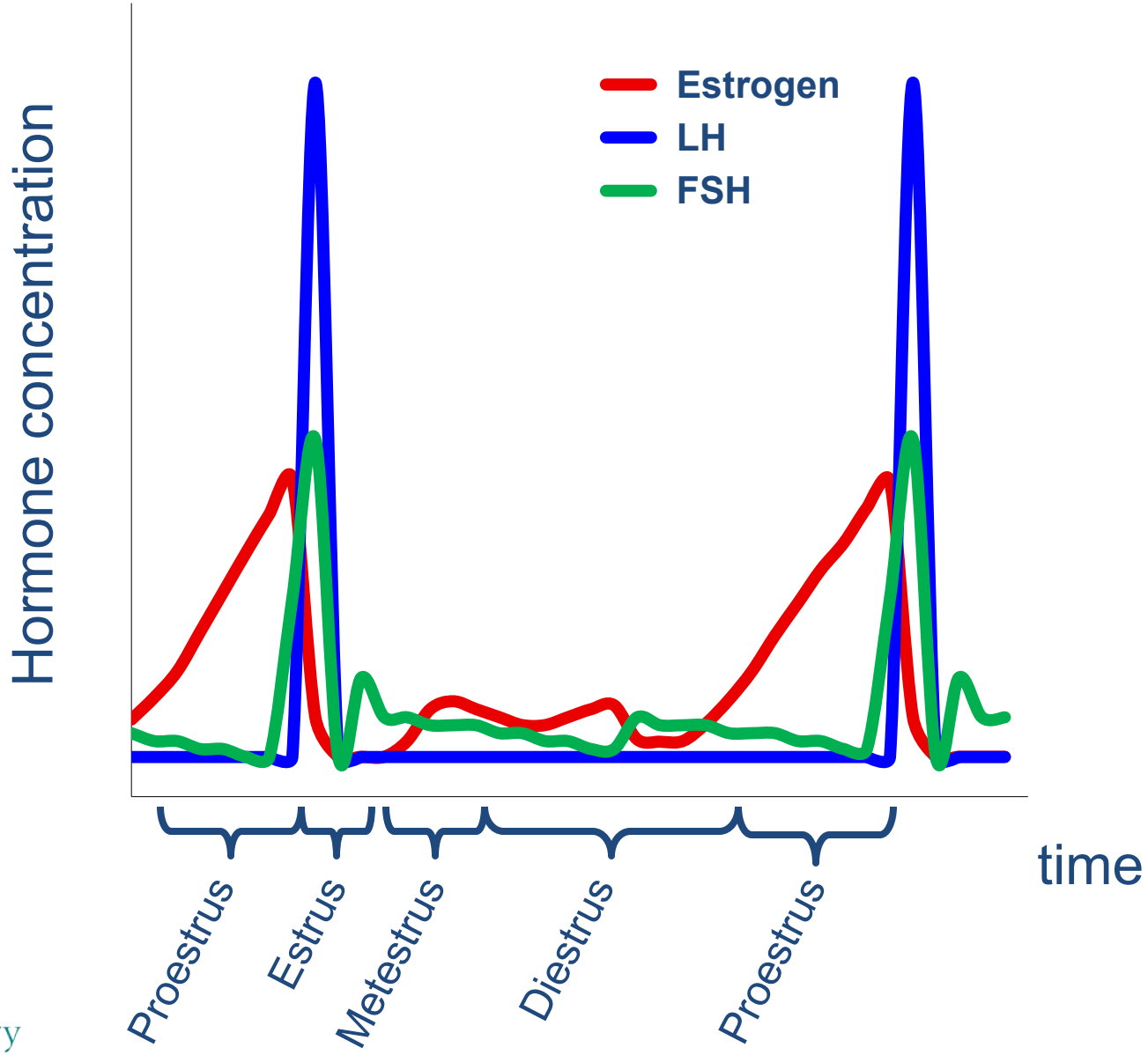






Champlin, Dorr, Gates. 1973. *Biol Reprod* 8(4):491-4. PMID: 4736343

Hormonal Changes During Estrous



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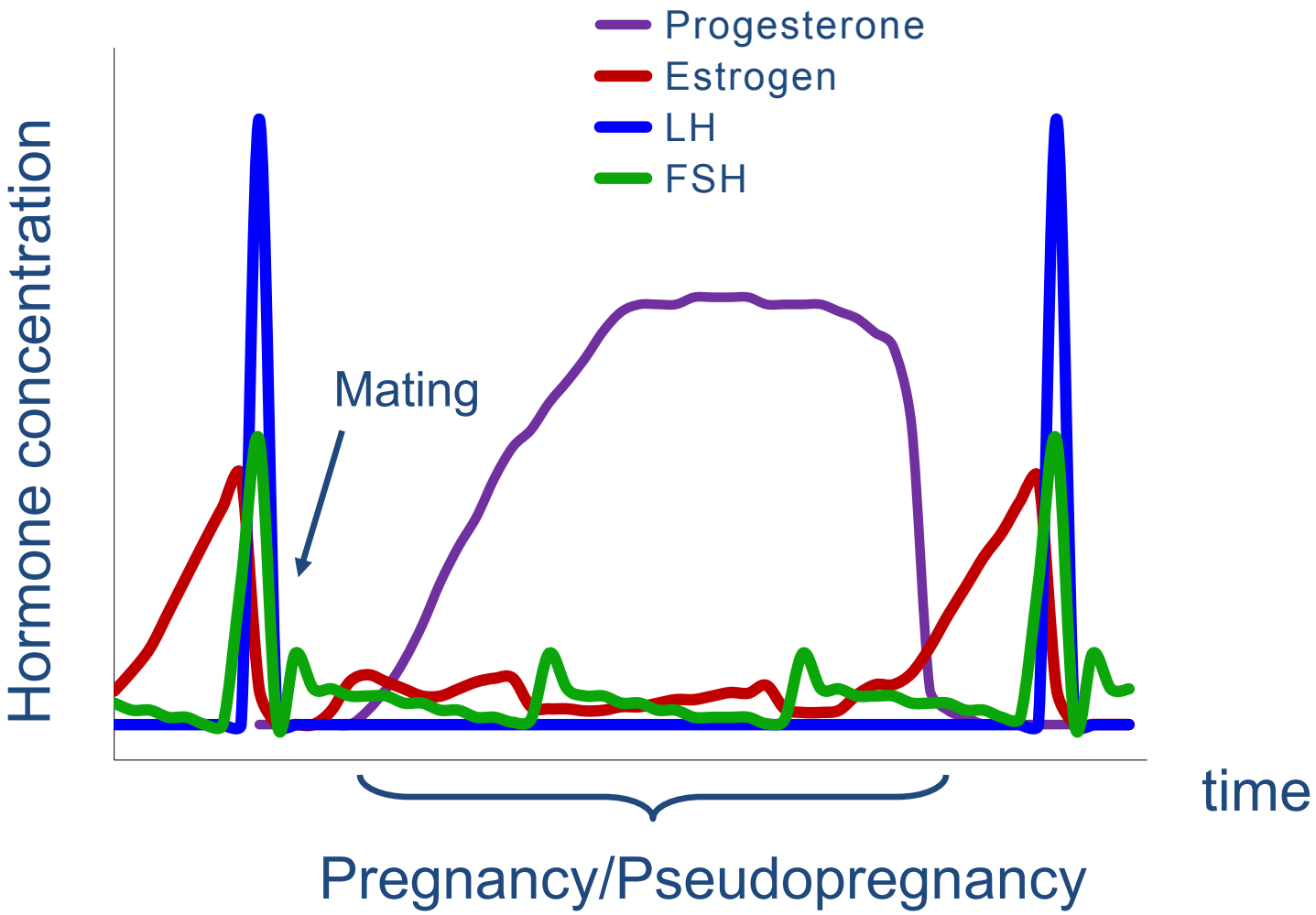
Estrous Cycle Interruption: Mating

Female in proestrus

Vaginal plug after 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



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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

Wesley K. Whitten

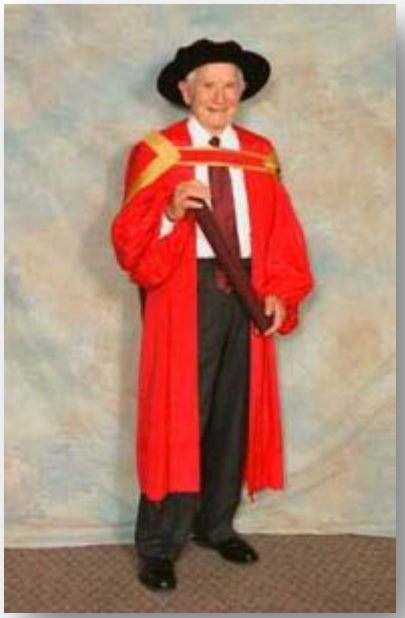

Photo: Memorial University of Newfoundland president's report

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Estrous Cycle Interruption: Pheromones

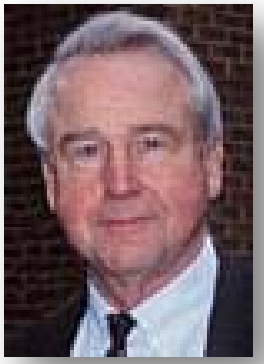
Bruce Effect - 1959

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

Hilda Margaret Bruce

Vandenbergh Effect - 1969

- Adult males accelerate puberty in pre-pubertal females



JG Vandenbergh

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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

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

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Silver LM. 1995. Mouse Genetics: Concepts and Applications
Oxford University Press. www.informatics.jax.org/silver/

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

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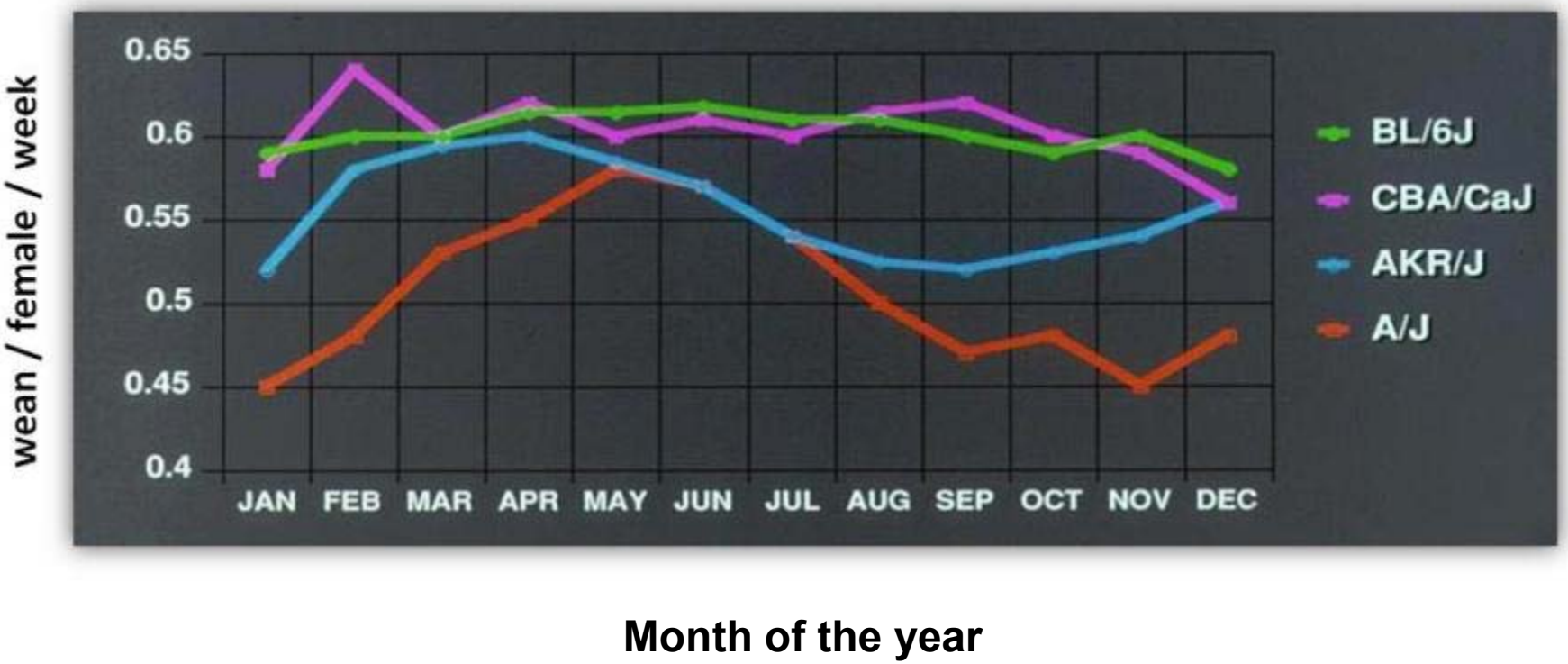
Seasonal Variation in Inbred Mice



Month of the year

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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)

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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

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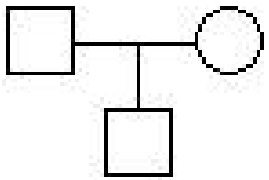
www.jaxmice.jax.org/support/husbandry/index

Troubleshooting Non-Reproductive Mice

My mice

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



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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?

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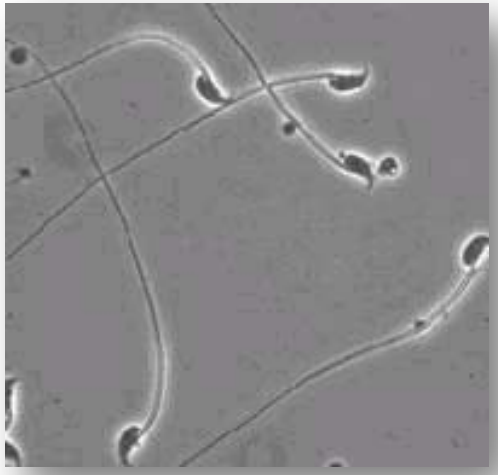
Strategies—Non-Productive Males

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



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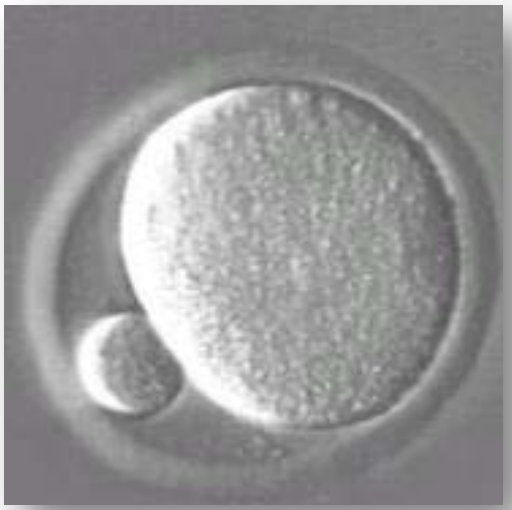
Strategies—Non-Productive Females

- 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



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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

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

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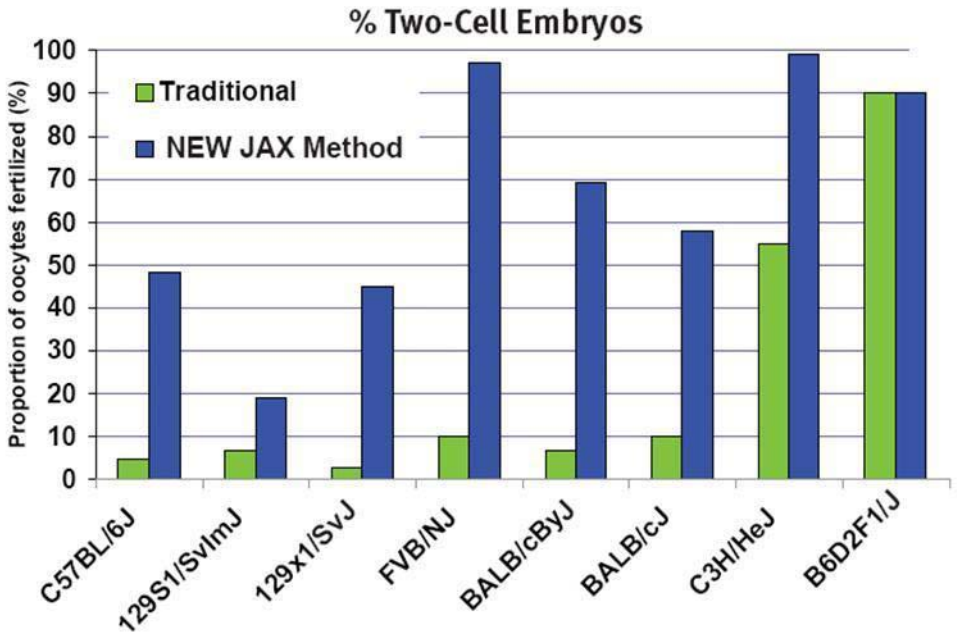
Cryopreservation

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?

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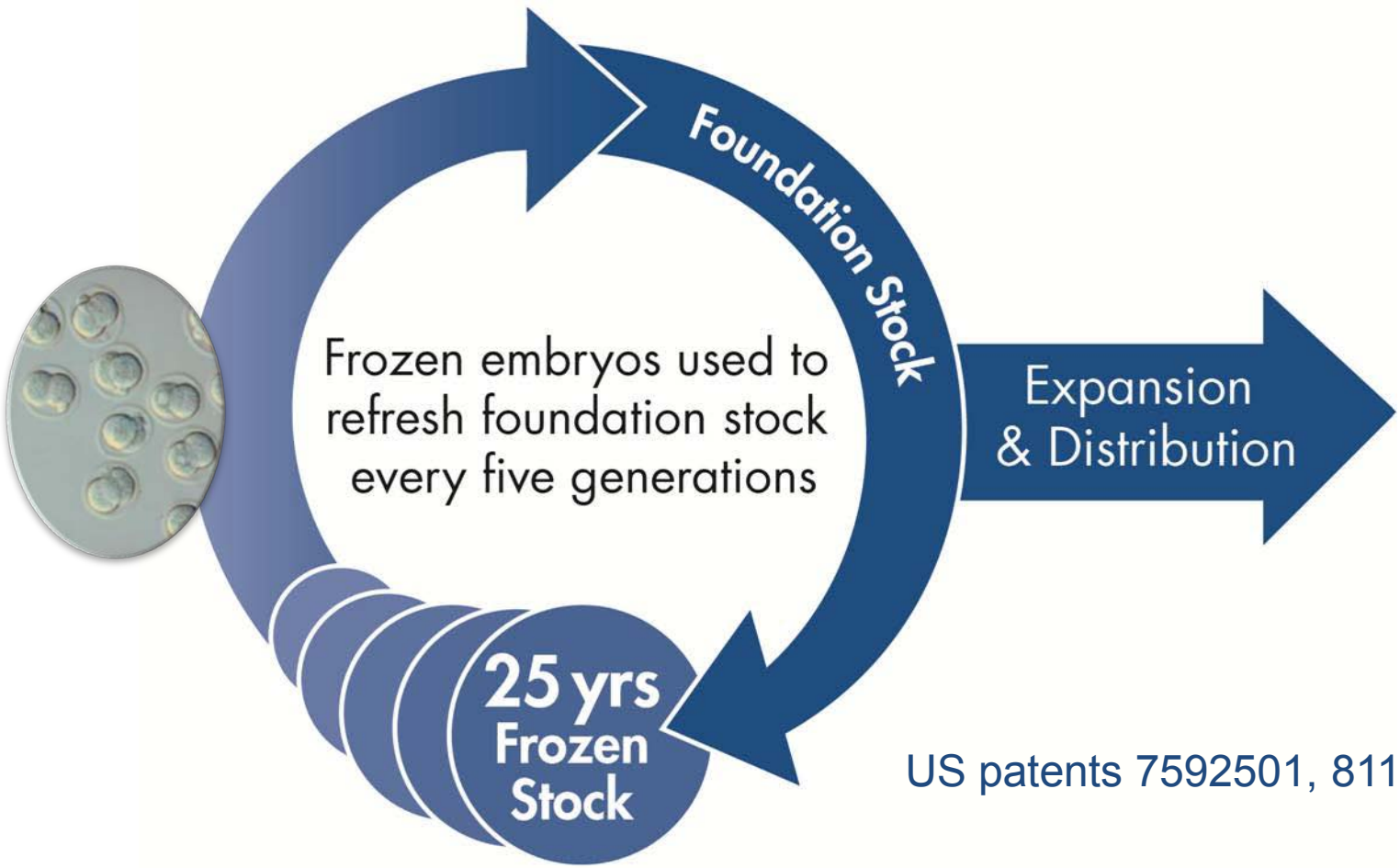
The Jackson Laboratory Genetic Stability Program



US patents 7592501, 8110721

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www.jaxmice.jax.org/genetichealth/stability

GSP Genetic Stability Program

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 & *in vitro* fertilization
  - Ovarian transplantation
  - Hysterectomy derivation
- Successfully rescued ~100 strains from extinction
- Call us before it is too late!

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