Mating techniques

- For successful mating, you need to pair a male with a female. . .

Mating techniques

- Determine the age of your mice
  - Minimum breeding age:
    - males: 35-42 days
    - females: 21 days
  - Maximum age for FIRST breeding:
    - males and females: 6 months

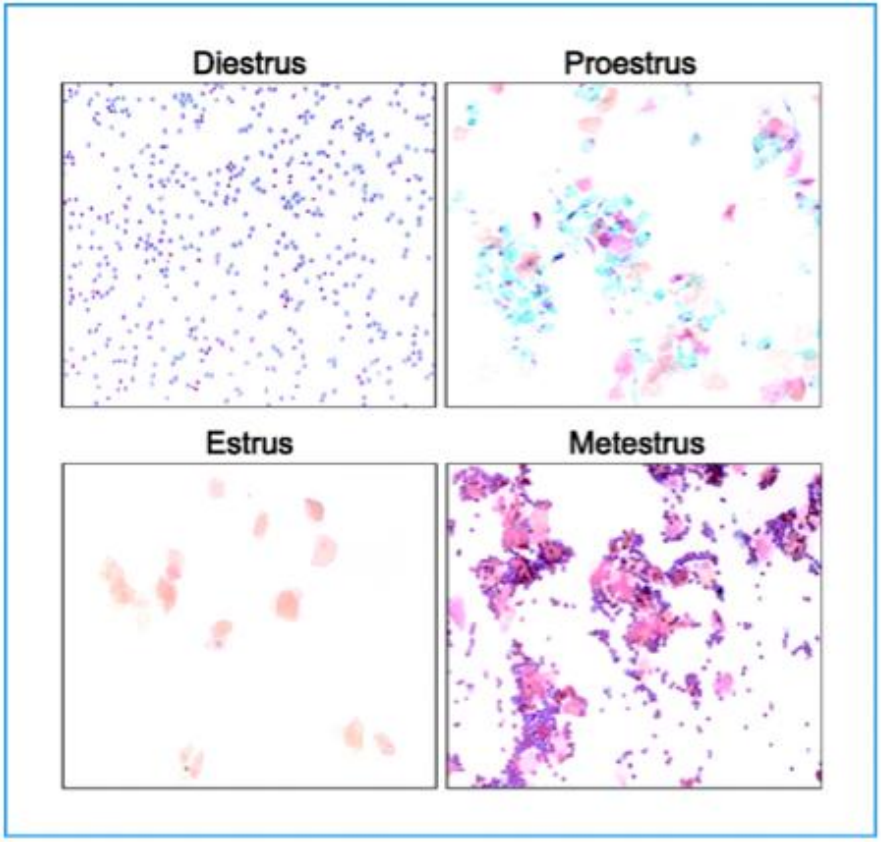
Mating techniques

- Questions:
  - Do I just put them together and wait?
  - Is there a way to insure that they mate?
  - How do I know if they have mated?
  - What if they've been together for a long time and I still have no pups?

Mating techniques

1) How do I know my female is ready to mate?
   4-day estrous cycle: can check for estrous using vaginal washes:

Changes in vaginal cytology associated with the different stages of the estrus cycle.



Hong H et al. Cancer Res 2010;70:221-228

Mating techniques

Is there a way to induce estrous?

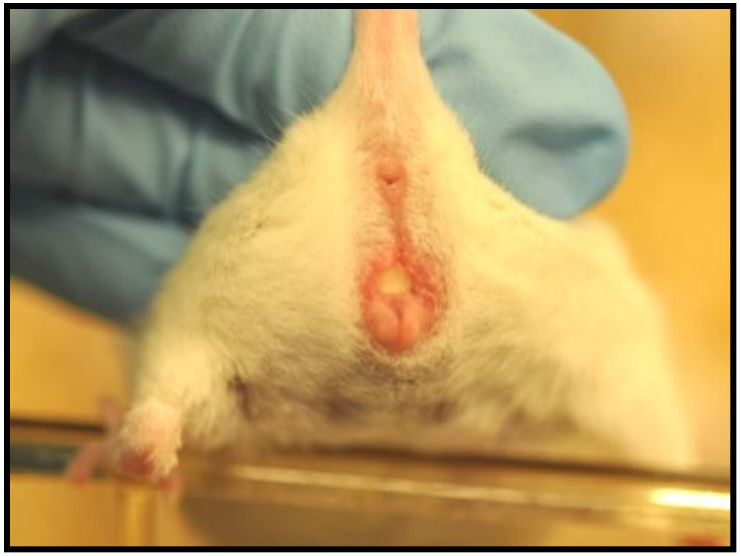
In young females, superovulation can be used:

Day 0: PMSG (typically 5IU/female)

Day 2: HCG (typically 5IU/female)

Mate immediately after HCG injection.

Day 3: Check for a vaginal plug, this will be day 0.5 pc



Mating techniques

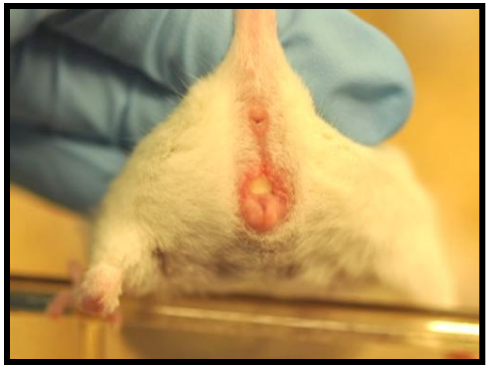
Is there a way to induce estrous?

In older females, use the Lee-Boot effect and Whitten effect to induce estrous:

House females in a fairly large group (harem mating--crowded—check with your veterinarian for approval) for up to 2 weeks; check for lack of estrous (the Lee-Boot effect).

When the females are all anestrous, add dirty bedding from a male's cage to induce estrous within 4-8 days; mate upon observation of estrous (the Whitten effect).

Check for a vaginal plug, this will be day 0.5 pc



Mating techniques

Common errors with mating:

1) Putting too many females with one male.

2) Finding a plug and noting the day for a timed mating, but leaving the female with the male.

3) Leaving the mating together, but not checking plugs on the weekends.

Common errors with mating:

4) Assuming the female is not pregnant after 8-10 days, and putting her with another male. (Bruce effect)

5) Depending on mating pairs that are too young or too old.

6) Leaving an unproductive mating together too long.

7) Either waiting too long to do something, or changing things too soon. . .

You need to pay attention and understand your mice; this is your research future!

Let’s say that you are the perfect postdoc/researcher, but there is still nothing.

What do you do?????

Troubleshooting

Troubleshooting

40 days and counting. . . NO PUPS!

Infertility?

Cannibalism?

Infection?

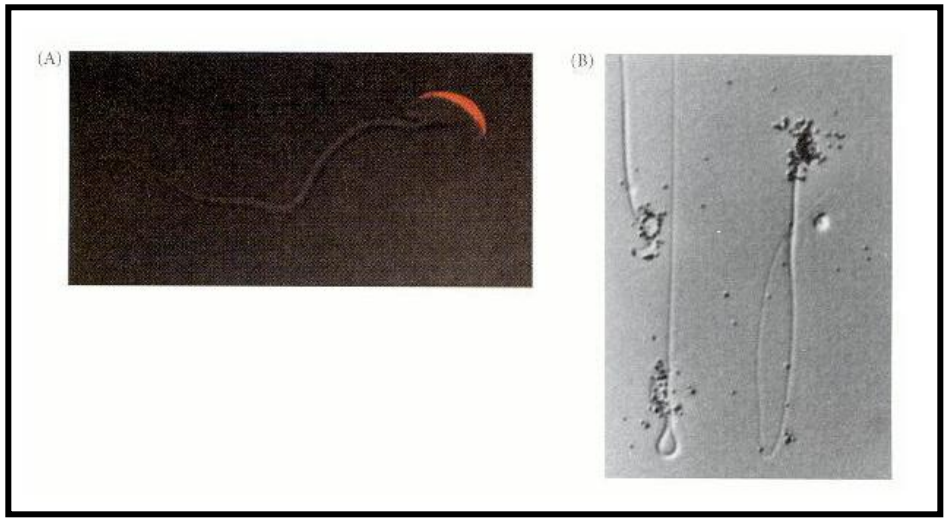
What else????

Infertility in male due to sperm problems. (Most common problem)

Set up timed matings and check for plugs. If no pups within 19 days, suspect infertility.

Set up the male with a superovulated, wild type female. Sacrifice after mating and look for fertilized oocytes.

Take another plugged female and isolate sperm from the vaginal tract. Look for motility under a high power scope.



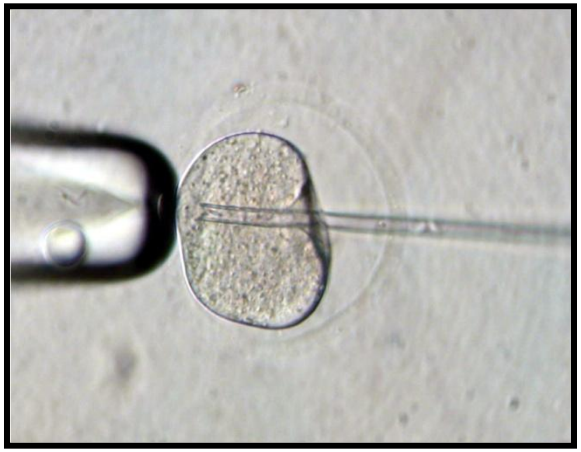
(A photograph by F. Suzuki, M. Toyota, J. Maekawa, J. Bleil and J. Cheng, courtesy of J. Bleil; B from Bleil and Wassarman 1986, photograph courtesy of the authors.)From: Recognition of Egg and Sperm Developmental Biology. 6th edition.Gilbert SF.Sunderland (MA)Sinauer Associates; 2000.Copyright © 2000, Sinauer Associates.NCBI Bookshelf.

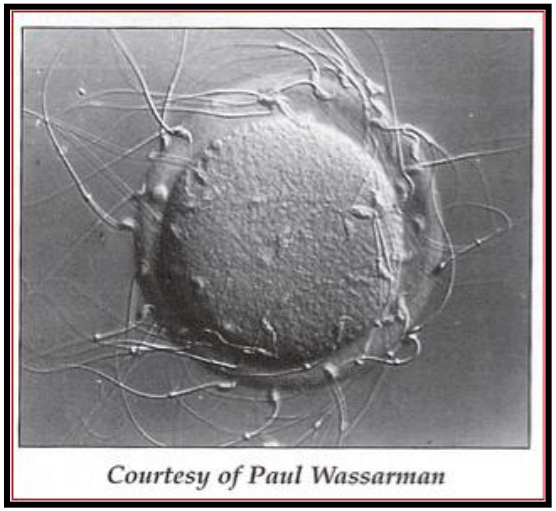
Infertility in male due to sperm problems--Treatment

If there is sperm, then ICSI can be used to generate additional pups. This requires special equipment and expertise.

If there is weakly motile sperm or reduced numbers of sperm, use IVF or IVF with zona thinning.

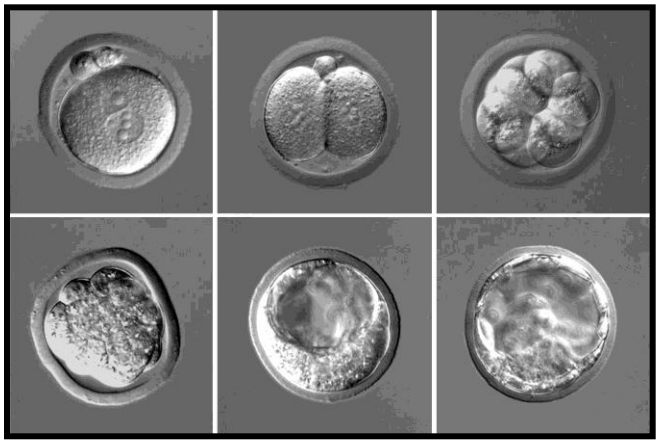
If there is no sperm, then carry the gene through the female.





Courtesy of Paul Wassarman

Troubleshooting



Infertility in female due to age or ovulation problems.

Check to see if there is an estrous cycle.

Attempt to superovulate the female, and then mate and check for a copulation plug. Expect to see pregnancy within 14 days.

Attempt to superovulate the female, and then obtain 1 or both oviducts; isolate oocytes for transfer into a pseudo pregnant female.

Perform ovary transfer into a younger female of the same strain background.

My female isn't too old, and she's had other litters, but I'm not getting pups any longer. . .

Has there been a change in the caretaker?

Where is the cage located?

Has there been a change in type of caging?

My female isn't too old, and she's had other litters, but I'm not getting pups any longer. . .

Are the lights going off at night?

What else has been changed in the room?

Is there nearby construction?

Troubleshooting

My female plugs and is pregnant but there are never any pups.

Cannibalism: Check for pup pieces (you can genotype these).

Bruce effect: Is she spontaneously aborting the pups?

Hydration: Is the female aborting due to dehydration? Other affected females in the room will also be cannibalizing pups.

Pre/Post	Date	Fert. eggs	impl. eggs	#pseudos	#preg.	# births	births/preg. fem
Pre	April01-Aug01		4296			217	5.05%
	Averages:	146.12	107.40	3.21	1.20	3.92	2.47
POST	Aug01-Jun02		*7595*			1086	14.30%
	Averages:	187.02	128.32	4.31	3.21	18.41	5.53

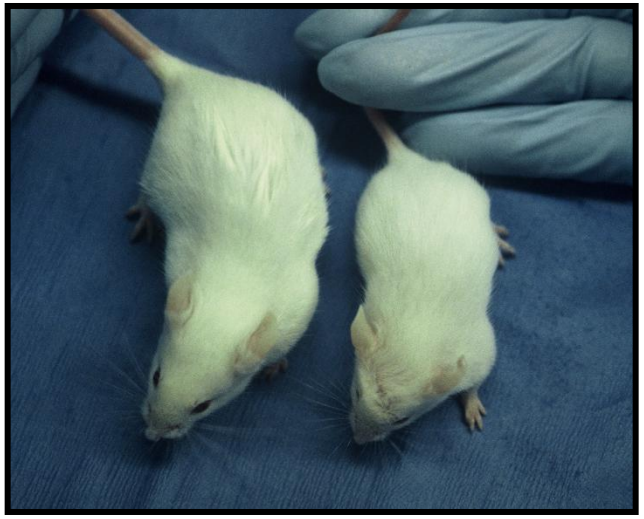
Troubleshooting

My female plugs and is pregnant but there are never any pups.

My female isn't too old, and she's had other litters, but I'm not getting pups any longer. . .

MAYBE THIS IS YOUR PHENOTYPE!

Strain effects, health issues and phenotypes



How can you tell?

Strain Issues:

C57BL/6:

- microophthalmia
- hydrocephalus
- prone to cannibalism

FVB:

- retinopathies
- stereotypies
- audiogenic seizures

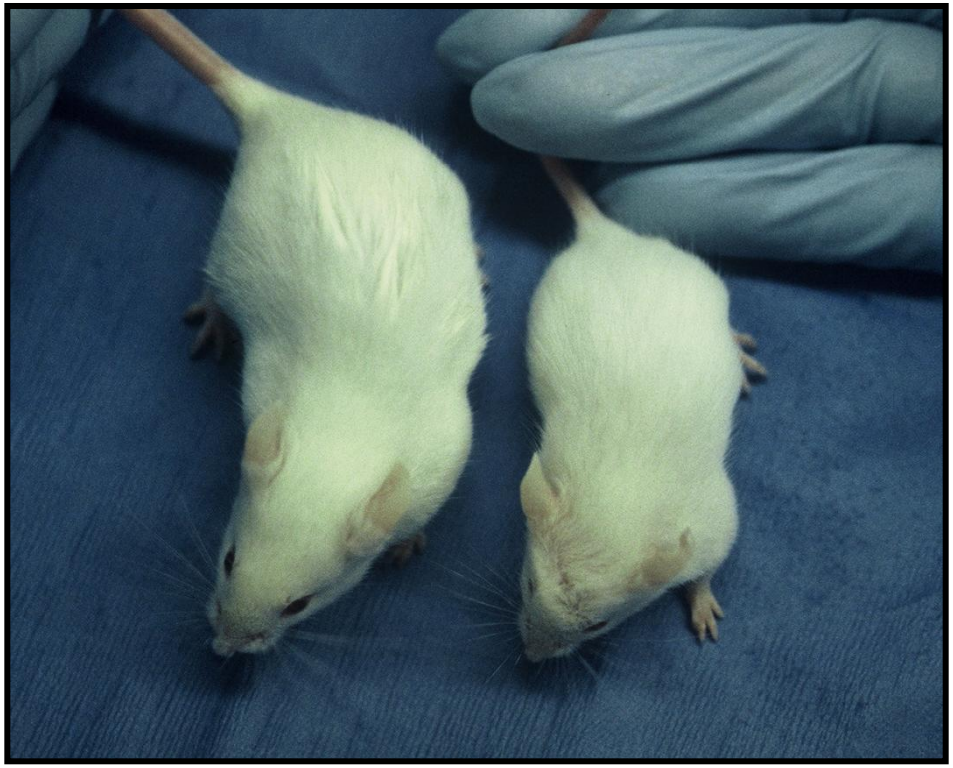
BALB/c:

- retinopathies
- males of one substrain demonstrate only 30% sperm fertility.

Health Issues:

- malocclusions (mice need to gnaw)
- eye infection presenting as microophthalmia
- deafness
- seizures
- stereotypies
- prolapses (penile and rectal)
- dystocia
- runting

Strain effects, health issues and phenotypes



Runting or dwarfism?

Strain effects, health issues and phenotypes

Barbering or SKH?

Strain effects, health issues and phenotypes

Dermatitis or erosion phenotype?

Problem:

PI has a line of mutant mice that she has characterized as embryonic lethal. After rederivation into the barrier facility, the homozygotes begin to survive. Why?

Solution:

The mutant line is a knock out for STAT3. They were rederived in from a facility with MHV.

Problem:

PI has a line of transgenic mice to test for memory and learning using a Morris water maze. The mice are in an FVB background. Will this work? Why or why not?

Solution:

FVB mice are blind as adults, and the Morris water maze depends on visual cues. This will not work.

Do we ever consider the ethical issues?

Search for alternate models?

Re-making an already existing mouse line?

- -genetic background, refusal to share

Generating sick animals

- Death as an endpoint?
- What is the humane endpoint?
- Tumor size? Ulcerated tumors?

It’s Complicated!

Mice are easy? Not necessarily.

You need to understand your mice and your project.

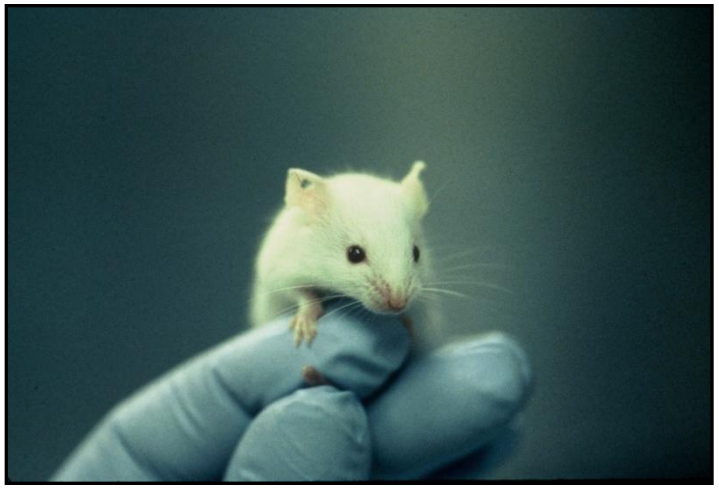
Most of all, you need to know who to ask when things don’t work out as planned.

The Mice Know!

They are an investment.

Pay attention.

Take care of them!



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