

Breeding Troubleshooting Tips

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The following tips will help you identify reasons your colony breeding yield may be below expectations. Use this as one more instrument in your toolbox to help you work out your breeding problems.

- ❑ What have the **room environmental conditions** been in this room during the times of deaths or breeding problems?

TIP: Think hi/lo (large fluctuations) in temperature and humidity, light timers not working well, and lights staying on 24 hours a day may have profound effect on reproduction, and in the case of the latter, animal may stop breeding altogether.

- ❑ **Supplement enrichment**

TIP: e.g. Red-tinged igloos, Shepherd Shacks extra Nestlets, EnviroDri, chewing sticks, treats, etc.? Keep in mind that in some strains (esp. among males) enrichment (esp. rid items) may lead to intra cage competition and fighting. Appropriate nesting material and/or shelters allow dams to keep neonates warm.

- ❑ **Vibration problems** in the facility, including that caused by nearby construction?

TIP: Consider anti vibration pads under racks. Ideal pad material is able to match the vibration source frequency & wavelength. Consider moving racks to a room further away from the source of vibration.

- ❑ **Location of cage in reference to the rack blower, computers, computer/TV monitors, animal transfer station, etc.**

TIP: The closer to those items, the greater the sound and vibration, and effect on reproduction.

- ❑ **Intermittent vs constant noise**

TIP: On/off noises can be stressful to rodents and affect reproduction, whereas constant or regular noises allow rodents to adapt. Some facilities leave biosafety cabinets, cage changing hoods and other equipment on all the time to avoid the irregular turning on and off of such equipment.

- ❑ **Volume of speaking, laughing, noisy keychains, loud equipment and cage docking**

TIP: The louder the volume the greater the effect on reproduction. When rodents are present, speak in soft voice, avoid loud joking/laughing, flip metal cage cards quietly, dock cages in the rack gently.

- ❑ Are you **moving animal cages from room to room using light or plastic carts**?

TIP: The plastic/lighter carts vibrate more. Consider pneumatic wheels to absorb transportation vibration.

- ❑ **Lubrication of equipment** that produce squeaky noise, bumping and vibration

TIP: Be sure all doors and cart wheels are lubricated to minimize noise and movement.

❑ **Location of breeding cage in reference to cages with sexually mature males**

TIP: The sense of olfaction is a key component to rodent behavior. Pheromones in particular can drive behavior and breeding. Certain sensitive strains and poor breeders in close proximity to sexually mature males (especially if cages are not on individually ventilated racks) may respond poorly to breeding. In such cases, avoid keeping breeding cages next to or in close proximity to a cage with sexually mature males. Also, avoid contact with bedding from such males when working with breeder cages.

❑ Other scents such as **disinfectants** and other **chemicals**.

TIP: Certain disinfectants and chemicals may affect reproduction. Consider all chemicals being used and consider using a reevaluate the disinfectant being used where animals are housed.

❑ **Energy dense supplementation** may help improve pup yield

TIPS: Consider the following supplementation

- ❑ Bacon Softies (www.bio-Serv.com).
- ❑ DietGel Prenatal 9 (<https://www.clearh2o.com/product/dietgel-prenatal/>). A high protein/high calorie supplement for lactating and breeding rodents
- ❑ DietGel Boost (<http://clearh2o.com/research-products/dietgel/dietgel-boost-2.html>): High calorie supplement to quickly move pups forward.
- ❑ Mix 50:50 with powder rodent chow and moisten with water for weak pups or mothers in needs of extra energy.

❑ What is the **fat and protein % of the diet** they are on?

TIP: e.g., some strains, such as BALB/c mice tend to have higher fat requirements than other strains for breeding. In this case consider a higher fat chow. In other strains, you may need to actually decrease fat content, but in most strains, mice that are too fat or too thin will typically breed less efficiently.

❑ What **breeding scheme** are you using (timed? monogamous, trio, harem?)

TIP: Some strains do breed more efficiently with a particular scheme or the other. Some do better if another adult is in the cage. Some don't. Know your strain.

❑ Are **males taken to the female cage or are females taken to the male cage?**

TIP: Typically, the female should be taken to the male's cage.

❑ Do you need to temporarily **halt breeding and separate** animals?

TIP: If you separate males and females, keep in mind that typically you may be able to bring females back together, but males will tend to fight with each other.

❑ **How old are the males? How old are the females?**

TIP: Older animals lose reproductive yield. Generally (although this is strain dependent), most strains productivity sharply decline after 9 months of age. If an older male must be used, replacing older breeding females for younger ones may encourage the older male to breed.

- ❑ Is this a **shared room** or is it assigned to one PI only?
TIP: Personnel traffic is disruptive to breeding and shared room typically have more traffic.
- ❑ **Who goes into the room? Are practices consistent** (thus importance of good SOPs)?
TIP: Inconsistent practices, too many people, exposure to new personnel or novel activities lead to stress and can affect reproduction.
- ❑ How well does personnel breeding animals **understand breeding**?
Staff working with breeding colonies should be trained on the genetics of your breeding program and appropriate record keeping methods.
- ❑ What's the **strain background** of these mice?
TIP: The particular background may have a known history of reproductive performance issues. Have you searched breeding issues related to your particular strain?
- ❑ **Age/weight at weaning**
TIP: Using the 10-gram weight could help in deciding when to wean. Typically, once a pup reaches 10 grams, it is ready for weaning.
- ❑ Is the **male getting older and heavy, decreasing his ability to breed**?
TIP: Encourage weight loss by placing a running wheel in the cage.
- ❑ Is there an **overcrowding** issue? e.g., **multiple litters or generations**?
TIP: When multiple generations exist in a cage, the older pups may be trampling younger pups. Mom may not be able to sustain both litters. Overcrowded conditions may lead to cannibalism.
- ❑ **Health issues**
TIP: Certain pathogens may affect reproductive efficiency. Know the health status of the colony.
- ❑ **Anatomical problems**
TIP: Have you conducted a physical exam for anatomical defects? Vaginal septum, imperforate vagina and preputial injury may be present in your colony and will affect breeding ability.
- ❑ Any **experimental manipulations during breeding, gestation or immediately postpartum** being conducted?
TIP: Consider delaying such manipulations for times that do not coincide with breeding, delivery and weaning.
- ❑ **Location of breeding cages within the room**
TIP: If cages are close to the door or area of high traffic, consider moving to the back of the room or an area of less traffic.
- ❑ **Light too intense in the room?**
TIP: Does light need to be attenuated or cages need to be placed on lower shelves of the rack? If on top rows of the rack, consider moving cages to the bottom rows to minimize light intensity.
- ❑ **Type of light in animal holding room**
TIP: Fluorescent lighting produces ultrasonic noise, which can be perceived by the mice. Consider shielding the lights with a solid cover (not open mesh).

Is the dark cycle being disrupted?

TIP: Personnel entering the animal holding room during the dark cycle is a common cause of breeding failure. Ask your facility manager to look into their access monitoring system to determine if individuals are entering the room after lights go off.

Electronic timers, temperature and humidity sensors

TIP: These should be kept at least one meter away from animals or they should be shielded.

Does the room have **audiogenic motion** sensors?

TIP: Replace them with passive infrared motion detectors.

Seasonal breeding depression

TIP: Lows in breeding yield is often noted during the late fall and winter months. Plan accordingly and if breeding needs to remain consistent throughout the year, consider increasing the number of breeders during this time.

Daily checks may affect reproductive yield

TIP: Perform daily checks using the least stressful method. Consider giving mice a veterinary-approved positive reward such as a sunflower seed treat during checks.

Problems with maternal care

TIP: If maternal care is an issue, consider pup fostering to outbred or hybrid moms, which tend to display strong maternal instincts.

Genetic factors?

TIP: It may be necessary to backcross or breed to wild type to maintain/revive the line. Important to keep breeders from previous generation until certain next generation will breed and have desired genotype.

Is colony management done properly?

TIP: A colony well managed allows the researcher to know when to cull breeders that are having performance or genetic issues. It may provide insight as to what time of the year breeding efficiency is highest or lowest.

Cannibalism?

Are cages being left undisturbed 2-3 days before parturition and at least 3-5 days post-partum?

TIP: This time may be longer for certain strains and may be a key component to prevent cannibalism and neglect by mother. Some strains may require longer undisturbed times.

Who is doing the cannibalism? Mom? Surrogate? Another cage mate?

TIP: Identify the offender.

First-time mothers or experienced mothers?

TIP: Often first-time mothers cannibalize but will not subsequent litters.

Decreasing the dark cycle (which is when the mice are most active)

TIP: Decreasing the dark cycle by 2 hours may decrease cannibalism, e.g. 14 hr light/10 hr dark may help.

Are males kept in the cage during the peri-partum period?

TIP: Males may be the guilty party, however with some strains males may actually help raise the young.

Is there another female in the cage to help raise pups during this time?

TIP: Additional females may help in some strains.

Are personnel coming into the room during the dark cycle?

TIP: Disruption of the light cycle, especially at the start of the dark cycle, when most mice copulate should be avoided.

Noise or excessive traffic in the room (or hallway)?

TIP: When rodents are present, speak in a soft voice, avoid loud joking/laughing, flip metal cage cards quietly, dock cages in the rack gently.

Anybody coming into the room that wears **perfume** or even **lotion** (such as hand lotion)?

TIP: Implement a no-perfume policy in the facility.

Extra enrichment

TIP: Additional enrichment may help minimize cannibalism.

Love Mash™ Rodent Reproductive Diet

TIP: This is a *Bio-Serv* product that when given to pregnant rodents it has shown to prevent cannibalism of litters in some dams.

Is the entire cage changed?

TIP: Consider leaving some of the old cage material, in particular nesting material in the new clean cage. Old odors/pheromones help keep the dams more calm.