

Clear **H<sub>2</sub>O**<sup>®</sup>

# Best Practices for Rodent Colony Planning, Breeding Support, and Pup Health



When planning for a breeding colony, there are key methods that support the fertility of rodents and mating. Factors must be taken into consideration to ensure a stress-free environment that includes enrichment and supportive nutrition. When conditions are not optimal for breeding, such as deficient nutrition, issues can arise that reduce productivity and/or threaten the health and performance of the pups.

To better understand best practices for breeding programs among laboratory animal professionals, ClearH<sub>2</sub>O conducted a survey entitled "Breeding Support and Pup Health" among AALAS members in March 2019 to gain their insights on the challenges they encounter during the various stages of the breeding process. Issues identified, as indicated in Chart 1 below, include poor mating, dystocia, poor lactation, small litters, runts, cannibalism, weanling weight, etc. Throughout this guide, we will examine ways to not only mitigate these challenges, but improve survival rates as well as breeder and pup health through the breeding process.

# Overall Breeding Difficulties

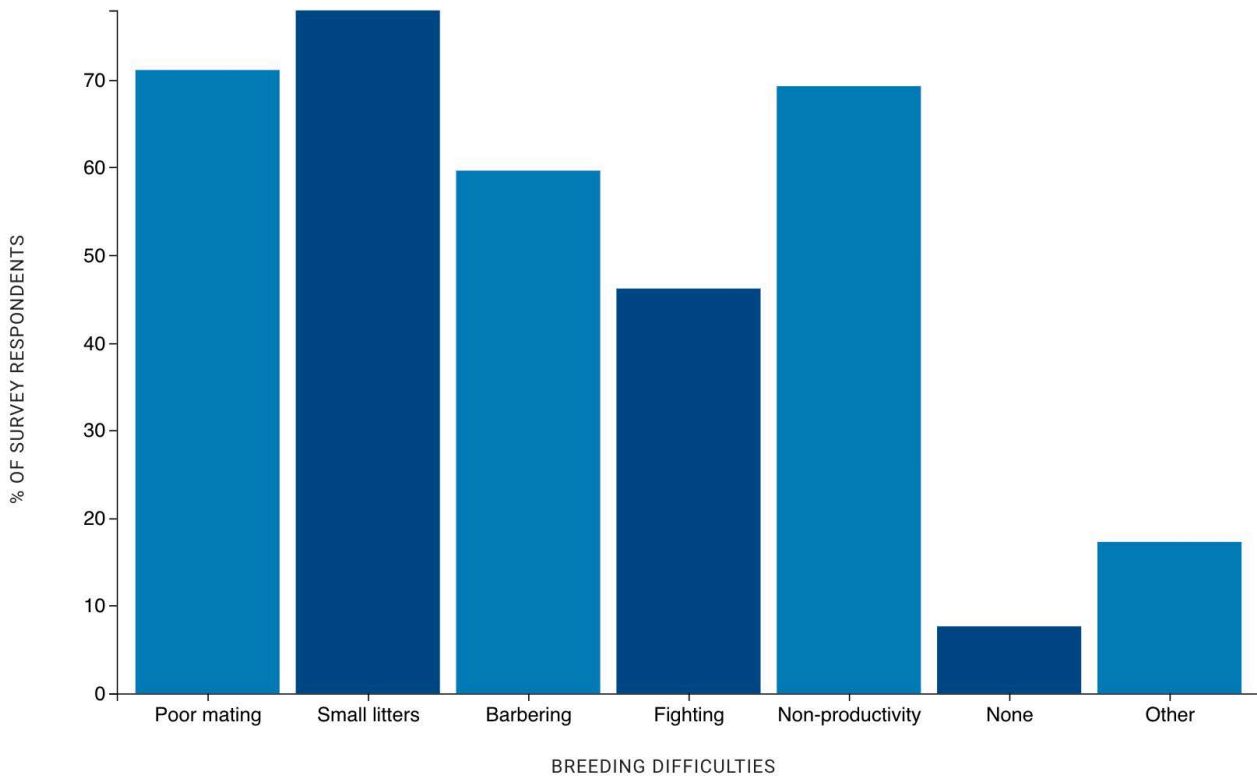


Chart 1: This chart shows common difficulties encountered throughout the overall breeding process by respondents of the ClearH<sub>2</sub>O survey, "Breeding Support and Pup Health", which was sent to AALAS members in March 2019. This guide will provide researchers and laboratory animal professionals with guidance on best practices for breeding rodents, along with how to mitigate common challenges, including those influenced by nutrition, from fertility to weaning.

# Environmental Factors

There are several ways to make the environment conducive for breeding that will keep rodents stress-free and improve performance for optimal outcomes <sup>1,2</sup>.

## Avoid Over-handling

Checking cages every 1-2 days is unnecessary and can cause stress to rodents, which may reduce breeding performance. It is recommended to change the cages no more than 1-2 times per week. Breeding cages and breeders should be handled gently, slowly, and quietly. Mice that are pregnant or have new litters should be handled as little as possible.

## Avoid Noise, Lights, and Smells

Mice have acute senses and can be greatly affected by noise, vibrations, or odors. To prevent distraction and stress, it is recommended that breeding cages be kept in the darkest location of the room, away from always-on lights such as exit signs, and away from doors and sinks where the traffic might be heavier. Adding white noise or background music may help cover any sudden sounds.

To avoid the transfer of pheromones and pathogens from one cage to the next, clean and disinfect forceps and gloves with 70% alcohol between cages. Perfumes or scented skincare products should not be worn, as they can impact the breeding process by stressing the mice.

## Provide Enrichment

Enrichment that provides something for rodents to hide in and play with is becoming standard practice in improving the health and welfare of laboratory animals. Even more so during breeding, providing shelters and nesting materials can reduce stress and improve breeding performance <sup>3</sup>. Providing foraging opportunities can also distract the breeders and reduce stress. The results from the "Breeding Support and Pup Health"

survey indicates that 27% of respondents use sunflower seeds as a foraging enrichment for their breeders—a way to distract them, reduce stress, and decrease food grinding <sup>4</sup>. ClearH<sub>2</sub>O's [DietGel™ Prenatal](#) contains sunflower seeds as foraging enrichment.

## Provide Supportive Nutrition

Nutrition has a major influence on reproduction <sup>5</sup>. The productivity of some strains of mice may be improved by using dietary supplements. Made for lactating and breeding rodents, ClearH<sub>2</sub>O's [DietGel™ Prenatal](#) is a high protein supplement that combines hydration and nutrition. More about the importance of diet is discussed in this guide.

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## Fertility

Prior to mating mice, the right conditions need to be in place and maintained throughout the generation cycle to increase the probability of pregnancy, healthy gestation, and pups. To ensure efficient breeding, major factors such as environment and diet must be taken into consideration. To maximize your colony production and to save time and precious resources, the following recommendations <sup>1</sup> can be helpful.

## Choosing the Right Age

Depending on strains, mice become sexually mature between 4-8 weeks of age. To increase the odds of mating, pick females that are 6-8 weeks old, as younger females



usually breed better than older ones. Mate them with older (12-16 weeks old) and/or experienced stud males. Avoid using older virgin females (15 weeks or older), and retire breeders at 6-8 months of age. Breeders that have not generated a litter, or have not brought a litter to wean age after two months of breeding are considered non-productive and should be removed from the colony.

## Isolating the Breeders

Males should be individually housed 1-2 weeks prior to mating to maximize their fertility, allowing the sperm count to fully recover after previous matings. In parallel, females should be housed in groups of 4-10 depending on the size of the cage. Due to the known Lee-Boot effect <sup>6</sup>, the release of an estrogen-dependent pheromone in the urine will synchronize or stop the females' estrous cycle. Once the females are placed in contact with a male or male pheromones from urine in the bedding, the estrus cycle will be induced and will resume (known as the Whitten effect <sup>6</sup>), maximizing the chances of mating and pregnancy.

## Providing an Appropriate Diet

Antioxidants—notably vitamin C, vitamin E, and folate (VitB9)—have been known to play an important role in reproduction. In females, folic acid in particular plays a critical role in several phases of reproductive health including maintenance of reproductive hormone levels by increasing progesterone levels, promoting ovulation, protecting against spontaneous abortions, and preventing birth defects. In males, folate deficiency is associated with decreased sperm counts and increased sperm DNA damage. ClearH<sub>2</sub>O's [DietGel™ Prenatal](#) contains 51.1mcg of folate/100g, formulated for lactating and breeding rodents.

When asked what type of dietary supplements are used in their breeding program, a cross section of respondents of the "Breeding Support and Pup Health" survey indicated using one or a combination of [HydroGel™](#) and [DietGel™](#) nutritional supplements. For example, one respondent stated that ***"DietGel™ is recommended by the veterinary staff to investigators who experience poor breeding performance or neonatal challenges."***

**– Ohio State University.**

# Mating

## Breeding Schemes

There are a number of breeding schemes that are employed in colony planning. Which one is used depends on many factors including strains, desired outcomes, and your Institutional Animal Care and Use Committee (IACUC) requirements.

- **Pair mating or monogamous matings consist of one female and one male:** This allows for easy identification of parents, lineage and pedigree. The litter sizes are also easily trackable. This scheme is recommended for strains with good fecundity to minimize overcrowding in cages.
- **Trio matings consist of one male with two females:** This is recommended for strains with average fecundity to maximize cage space. It also reduces the number of males to keep for breeders and produces more pups/male. The mothers help each other with feeding and raising the pups.
- **Harem matings consist of one male with three or more females:** This breeding scheme is recommended for difficult breeders because it produces the maximum number of pups per male and the pups receive the best care from multiple females. With trios and harem matings, overcrowding should be closely monitored—each dam and her pups must be removed to another cage if any animal welfare concerns are observed such as trampling, fighting, or filth. Fighting is identified as an issue by over 46% of respondents of the "Breeding Support and Pup Health" survey. Follow your IACUC requirements and facilities guidelines and policies concerning number of animals per cage.

- **Rotation matings consist of one male being rotated weekly between multiple female cages:** This scheme is useful to quickly establish a colony with only one or a limited number of males. For example, this situation that may occur when creating a new transgenic line. Once the male has been removed from the female cage, it should not be returned until after the pups are weaned.

**During trio and harem mating breeding schemes, welfare issues such as trampling, fighting, and filth can occur. Fighting was identified as an issue by over 46% of respondents of the “Breeding Support and Pup Health” survey.**

In all cases, it is recommended to bring the females to the male cage for the first mating to take advantage of the male pheromones in the cage and the Whitten effect. Transfer the females in the evening, as mice are more active and mate at night. Mice have a 4-5 day estrus cycle<sup>7</sup> and mate 4-6 hours into the dark cycle. Leave the male with their females at all times—doing so allows you to take advantage of the postpartum estrus, a period of 24 hours after parturition when females are fertile and can conceive again, so you can quickly expand your colony. After this period, the females will not be fertile until the pups are weaned. Daily monitoring at the end of pregnancy is required and the first litter must be weaned just before the second litter is born to avoid overcrowding and trampling of the neonates.



# Colony Management

There are a few ways to manage your colony depending on what you want to achieve. Below are some methods for colony management.

- **Timed pregnancy:** If you work on embryonic development, or need to study mice before birth, then the timed pregnancy approach is right for you<sup>8</sup>. Plan on 30% more females than needed to account for females that will not get pregnant. Put males and females together in the evening but remove the males in the morning. This will allow you to have all the females that do get pregnant at the same gestational stage. You can also check for vaginal plugs early in the morning. The presence of a vaginal plug indicates which females have actually mated, and have a chance for pregnancy. This defines day 0 of pregnancy, which can be verified by palpation on or after day 11.
- **Burst breeding:** If you need a large cohort of mice within 1-2 weeks of age, you can do a burst mating<sup>9</sup> by mating a large number of breeding units on the same day. Scale the number of breeders to the number of mice needed for the study. Take into account the performance of the strain (challenging, good, exceptional) and keep in mind that doing a study on only one sex doubles the size of your breeding colony.
- **Maintenance colony:** If you wish to have mice on hand for weekly experiments or just keep a small breeding colony to maintain the strain live on the shelf, a maintenance colony is the best solution. For maintenance, a colony of six breeding units, replacing one unit per month, gives you the most efficient performance. Keeping breeders of various ages produces a more consistent number of pups. Keep only the breeders you need for the next round will keep costs low.

# Gestation

The gestation period for laboratory mice is between 18-21 days depending on the strains. Pregnant mice, mice giving birth, or mice with a litter should be handled as little as possible, as they respond best to calm and infrequent handling.

During pregnancy, the daily requirement of iron increases tremendously<sup>9</sup>, reaching its highest levels needed in the third trimester. This is in response to the increased blood volume to assist in the growth of the fetus, placenta, and maternal tissues. Iron deficiency during pregnancy can lead to many issues related to fetal development, including anemia, low weight, growth retardation, neurologic impairments, compromised immune system, and more. ClearH<sub>2</sub>O's [DietGel™ Prenatal](#), formulated for breeding rodents, contains 2mg of iron/100g.

Survey respondents using dietary gel supplements in their breeding program observed an overall increase in productivity and improved reproductive performance, and reported that females receiving the dietary gel were less stressed, had improved lactation, recovered quickly from pregnancy, and were also quicker to mate again. The benefits were not only observed among the females, but also among the pups, where survey respondents using dietary gel supplements noted larger litters, healthier pups with higher weight, and increased pup survival.

***"Better coat condition, quicker to rebreed, better survivability of weanlings"***

***– Missouri University***

***"I've had good feedback from the labs with the DietGel™ Prenatal: we aren't losing a lot of pups." – Northwell Health***

***"Most of the time, the litters have a higher survival rate. We have also seen strains become more productive after being given supplemental feed."***

***– University of Oklahoma***

# Lactation

Once the pups are born, careful and infrequent handling are even more important to reduce as much stress as possible on the mothers. If you require a cage change with newborns, transfer part of the nesting and bedding to the new cage to maintain a familiar odor environment.

## High Energy Demand

Since the mouse achieves one third of its growth during lactation <sup>10</sup>, this imposes a high nutritional burden on the breeding females. Fatty acids—notably Omega-3, which is present in flaxseed oil and fish oil—are beneficial when provided during both pregnancy and lactation for early brain development of the embryos and pups. Omega-3 fatty acids also inhibit inflammation and bacterial growth in pregnant mice <sup>11</sup> and reduce preterm births, miscarriages, and stillbirths. ClearH<sub>2</sub>O's [DietGel™ Prenatal](#) contains fish oil and flax seeds, providing 15.2% fat which includes 2.2g Omega-3 fatty acids/100g.

## Cannibalism

Cannibalism can occur among all rodent strains, including inbred or genetically engineered mice (GEM). When that happens, the consequences on the colony can be devastating. With GEM, you can lose very precious (rare and expensive) animals. With a large scale production colony, cannibalism leads to inefficiency and higher costs. Cannibalism usually occurs within the first 96 hours after birth and some strains can eat up to 50% of their pups <sup>12</sup>. Results from the "Breeding Support and Pup Health" survey, as shown in Chart 2, indicated that almost 65% of respondents encountered cannibalism issues.

**A study from Indiana University with DBA/2 mice showed that [DietGel® 76A](#) increases the number of pups per litter, decreases cannibalism and increases the breeders' weight. <sup>13</sup>**

There are a number of factors that can provoke parents to eat their young. As described above, noises, cage changes, smells, and unfamiliar technicians can stress the mice and rats. Such disturbances to the parents might lead to neglect, death, and eating of the young <sup>14</sup>. Malnutrition or undernutrition during those high demands will intensify the deficiencies and might lead the female to eat their pups. In that case, cannibalistic behavior might result from a specific need in protein more than hunger <sup>15</sup>. ClearH<sub>2</sub>O's [DietGel™ Prenatal](#) contains 14.3g of protein/100g.

# Pup Health Challenges

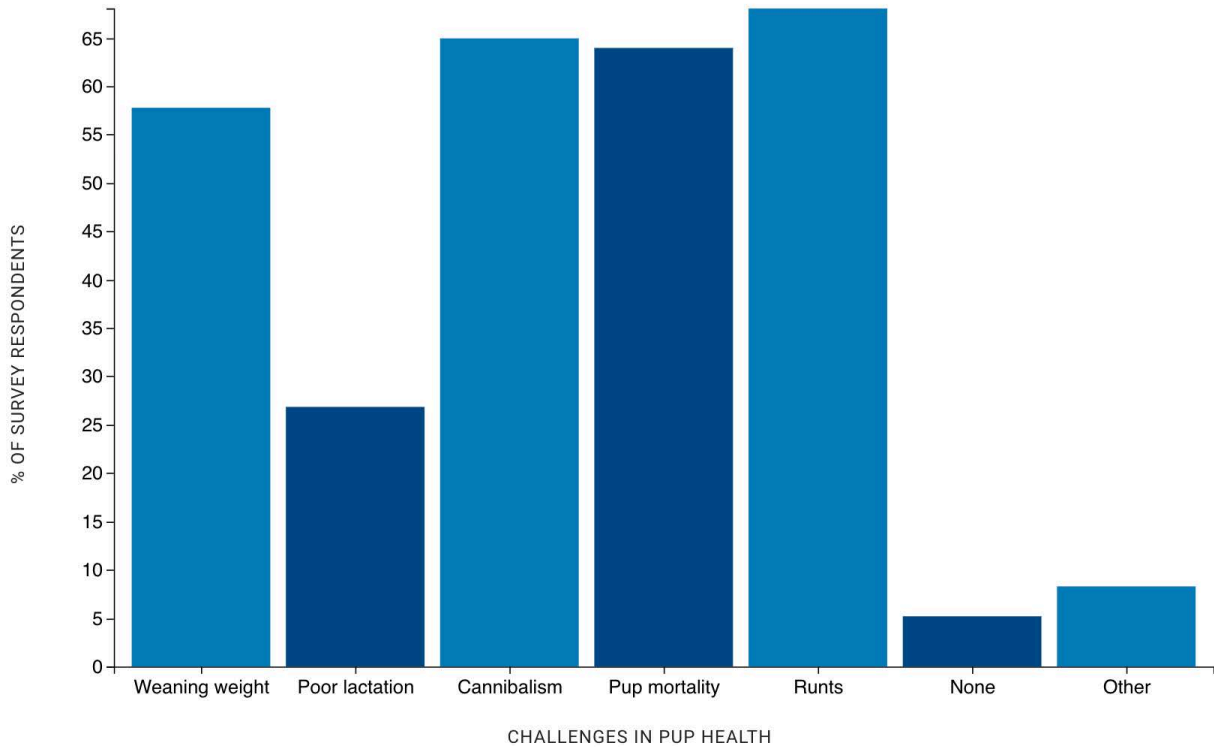


Chart 2: This chart shows the challenges related to the health of the pups encountered by respondents of the ClearH<sub>2</sub>O survey, "Breeding Support and Pup Health".

# Fostering

Fostering is a common method used to save pups when their moms are not able to take care of them. This can be due to various reasons including lack of nurturing, lactation defects, or cannibalism. Plan on breeding a foster unit of a well-known caring strain (such as CD1 or Swiss) at the same time as your strain of interest. When pups are born, discard as many natural pups from the recipient mom as the number of foster pups you want to add from the donor mom.

## ■ Weaning

### Weaning Process

In a laboratory, weaning refers to removing pups from their parents' cage. Generally, laboratory mice are weaned at 3-4 weeks of age. Most strains are mature enough to be weaned at 21 days of age and separated into same sex cages to avoid unwanted pregnancies. Some strains or some runt pups can benefit from extended weaning and spend an additional week with the mom. However, if you are keeping the breeders together, the female(s) might give birth to a second litter after 3 weeks. If you decide to keep the first litter longer than 3 weeks in the cage, some overcrowding and trampling of the little pups may occur. Make sure to check daily and remove older pups when able. Remove the weanlings at 21 days if possible and only keep the runt or smaller pups who will benefit from extra care in the cage with the mom. That will limit the number of animals in the cage for the breeding female to care for if she births a second litter.

# Weaning Cage Set Up

Follow your institution's Animal Care and Use Committee and Standard Operating Protocol. Many institutions, such as Emory University <sup>16</sup>, Johns Hopkins <sup>17</sup>, The University of Iowa <sup>18</sup>, University of Rochester <sup>19</sup>, and UCSF <sup>20</sup> require a specific weaning cage set up including water gel, moistened food, soft diet supplements, and/or nesting materials to help the animals transition to their new environment without the breeding female. Weanlings can indeed have a hard time finding food or water, using the Lixit, or reaching and chewing feed pellets, as indicated in Chart 3. A supplement at the bottom of the cage can make all the difference in the survival of these animals. ClearH<sub>2</sub>O's [DietGel™ 76A](#) is a complete soft diet supplement containing protein, fat, carbohydrates, fiber, vitamins, and minerals needed for the survival and growth of weanlings.

A study from Tufts University showed that providing C57BL/6 breeder cages with DietGel® 76A in addition to regular water and chow increased pup survival to wean age by 40%.<sup>21</sup> The pups also weighed an average 17% more than with standard chow only.

## Weaning Issues

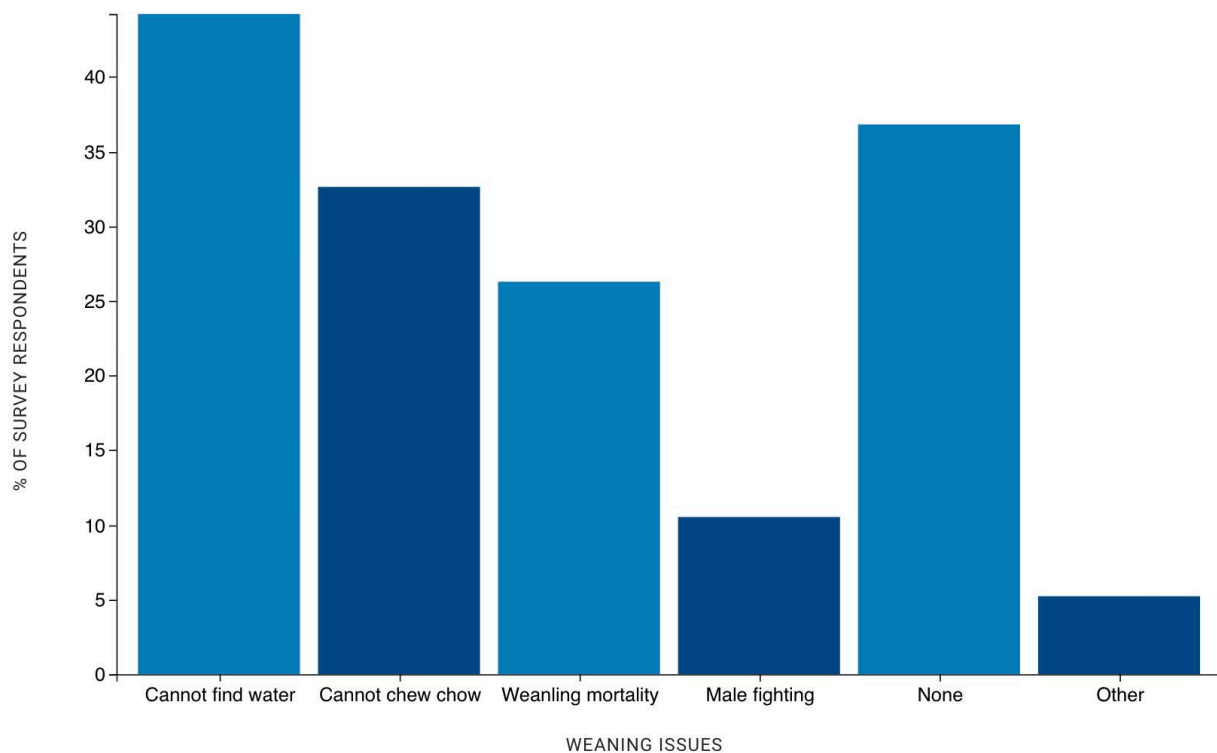




Chart 3: This chart shows which common issues respondents of the ClearH<sub>2</sub>O survey, "Breeding Support and Pup Health", face when weaning laboratory rodents.

From the survey, "Breeding Support and Pup Health", 24% and 42% of respondents, respectively reported that their institution's cage preparation requirements include hydration gel or moistened pellets at the bottom of the cage.<sup>22</sup> ClearH<sub>2</sub>O's [HydroGel™](#), [DietGel™ 76A](#), and [DietGel™ Boost](#) are effective supplements for weanlings. Respondents using these gels have reported that they help weanlings better acclimate to the cage setting, such as figuring out the Lixit or being able to reach and chew food pellets. The addition of hydration and dietary gel supplements provides essential support for weanling pups to better endure the critical first few days of transition during the weaning process. It gives them easier access to water and food when they are too small to reach the water bottle and wire rack filled with food pellets.

**Respondents reported that using dietary gel supplements increases weanling weights, survival, and provides a more consistent growth rate overall.**

*"Weanlings will put on weight quicker when using DietGel™ Boost. We lose less mice to dehydration when a water gel is available." - NYU*

*"DietGel™ Recovery has increased weanling survival." - Dartmouth College*

*"We observed better weanling survival when HydroGel™ and DietGel™ Boost supplementation is used." - University of Maryland*

# Malocclusions

Mice have two pairs of incisors that grow continually throughout their life, worn away through normal use and remain permanently sharp. Sometimes, because of misalignment of the jaw, or uneven wear of the teeth, a malocclusion, defined by an irregular alignment of the teeth, can occur quite commonly in laboratory mice<sup>23</sup>. If left untreated, the malocclusion can lead to malnutrition and premature death. Treatment consists in trimming the elongated teeth to provide temporary correction. As a long-term solution in a valuable transgenic mouse for example, the entire tooth can be removed, and soft diet must be given.

***"In the instances where animals have poorly developed, or cases of malocclusion this is a bridge to get them to a development point of dental development for them to be utilizing/efficiently ingesting normal pelleted diet." – University of Kentucky***

# Male Aggressive Behavior

Aggression is a dominance-related behavior and usually a male problem<sup>24</sup>. Standard recommendations are to keep male weanlings from the same litters together and not to combine males from different litters or from shipping boxes at reception. Males mark their territories with scent, so a complete cage change which eliminates all olfactory familiarity will mitigate aggression, with the exception of the nesting material—which when transferred to a new cage—seems to mitigate aggression. If needed, males can be single housed. If wounded, males can be given a pain reliever. ClearH<sub>2</sub>O's [MediGel™CPE](#) and [MediGel™ MLX](#) are complete dietary supplements with 5mg/kg carprofen and 5mg/kg meloxicam, respectively, for pain management.

# Gel Supplements

## Gel Dietary Supplements for Improved Breeding Outcomes

The results of the ClearH2O survey show that the addition of hydration and dietary gel supplements such as ClearH2O's [HydroGel™](#), [DietGel™ 76A](#), [DietGel™ Prenatal](#), and [DietGel™ Boost](#) provide essential support for both breeder performance and pup survival, growth, and health.

Delivered through highly-palatable gel formulations, these hydration and dietary supplements are a cost-effective solution that help laboratory animal professionals and researchers improve overall animal health and welfare while optimizing research and productivity. [HydroGel™](#) is the #1 choice of laboratory animal breeders worldwide.

Discover firsthand how ClearH2O's gel technology can help you achieve your research goals while improving the health and welfare of the animals in your care. Request a sample of ClearH2O products today.

## Request an Animal Research Product Sample

Are you a research veterinarian looking to improve the quality of life for animals in your care, lower labor costs, and gather more count, valuable research data? Request a complimentary sample of ClearH2O products today.



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# References

- 1 [Breeding strategies for Maintaining Colonies of Laboratory mice – A Jackson Laboratory Resource manual](#)
- 2 [5 reasons why your mice aren't breeding – The Jackson Laboratory – Dominique Kagele](#)
- 3 [Using environmental enrichment and nutritional supplementation to improve breeding success in rodents – LabAnimal 2016 - BioServ](#)
- 4 [Breeding and maintenance of an Mecp2-deficient mouse model of Rett syndrome – DG. Jugloff et al., Journal of Neurosciences methods 2006 \(PUBMED 16439027\)](#)
- 5 [Mouse Breeding and Colony Management – Current protocol in Mouse Biology - A. Ayadi et al., 2011 \(PUBMED 26068995\)](#)
- 6 [Reproduction and breeding in the Laboratory Rat – J. Lohmiller and S. Swing – Science Direct 2006](#)
- 7 [Mouse Genetics: Concepts and Applications – Lee Silver – Oxford university Press 1995](#)
- 8 [6 steps for setting up timed pregnant mice – The Jackson Laboratory – Jim Yeadon](#)

- 9 [Effect of Dietary Iron on Fetal Growth in Pregnant Mice – A. Hubbard et al., Comparative Medicine 2013 \(PUBMED 23582419\)](#)
- 10 [Nutrient Requirements of the Mouse – National Academies Press 1995](#)
- 11 [Omega-3 fatty acids suppress Fusobacterium nucleatum-induced placental inflammation originating from maternal endothelial cells – i. Garcia-So et al., JCI insight 2019 \(PUBMED 30728337\)](#)
- 12 [Use of Perphenazine to Control Cannibalism in DBA/1 Mice – D. Bart Carter et al., Comparative Medicine, 2002 \(PUBMED 12405639\)](#)
- 13 [Evaluation of Six Industry Diets on the Reproductive Success of DBA-2 Mice - J. Peveler and DL. Hickman, Poster](#)
- 14 [Cannibalism in Mice and Rats –W. Lane-Petter – Proceeding of the royal Society of Medicine 1968 \(PUBMED 5727015\)](#)
- 15 [Nutritional value of cannibalism and the role of starvation and nutrient imbalance for cannibalistic tendencies in a generalist predator – D. Mayntz and S. Toft – Journal of Animal Ecology 2006 \(PUBMED 16903066\)](#)
- 16 [IACUC – Weaning mice and rats – Emory university](#)
- 17 [IACUC – Management of Mouse Breeding Colonies – Johns Hopkins University](#)
- 18 [OAR Informational Sheet: New Weanling Procedure for Labs – The University of Iowa](#)
- 19 [Mouse Cage Density Policy – University of Rochester](#)
- 20 [IACUC - Standardized Care for Weanling mice - UCSF](#)

- 21** [The Effects of Two Different Supplemental Gels on C57BL/6J Pup Survival and Weights - K. Southwell and S. Perkins, Poster](#)
- 22** [IACUC Quick reference : Improving Mouse Breeding Success – C. Ferrecchia, H. Chum, and G. Lawson – University of California, Berkeley](#)
- 23** [Severe prognathic malocclusion – LabAnimal 2007](#)
- 24** [Reducing Aggression in Mice – Charles River technical Sheet](#)