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Development and Reproduction of the Laboratory Mouse

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Abstract

Successful breeding of the laboratory mouse (*Mus musculus*) is critical to the establishment and maintenance of a productive animal colony. Additionally, mouse embryos are frequently studied to answer questions about developmental processes. A wide variety of genetic tools now exist for regulating gene expression during mouse embryonic and postnatal development, which can help scientists to understand more about heritable diseases affecting human development.

This video provides an introduction to the reproduction and development of mice. In addition to clarifying the terminology used to describe developmental progression, the presentation reviews key stages of the mouse life cycle. First, major development events that take place in utero are described, with special attention given to the unique layout of early rodent embryos. Next, husbandry protocols are provided for postnatal mice, or pups, including the process of weaning, or removal of pups from their mother's cage. Since males and females must be separated at this stage to prevent unscheduled mating, the demonstration also reveals how to determine mouse sex. Subsequently, instructions are given for carrying out controlled mouse breeding, including screening for the copulatory plug, which is useful for precisely timed embryonic development. Finally, the video highlights strategies used to investigate the complex processes that govern mouse development, including the generation of genetically altered "knockout" mice.

Transcript

Mice are extremely valuable model organisms that continue to improve our understanding of human development and disease. Among mammals, mice have a high fecundity and rapid development, meaning that colonies can be quickly expanded.

Since development within the womb requires some specialized embryonic structures, the study of embryogenesis in a mammalian model is also more relevant to humans. In this video, we will discuss the stages of mouse reproduction and development, how to breed mice, and ways to apply mouse reproductive and developmental knowledge in the lab.

First let's talk a little bit about mouse reproduction. Like humans, early mouse development is internal, with gestation occurring within the uterine horns of the mother, who's called a "dam." However, unlike most of us, mice carry many fetuses at once, producing an average litter size of 10 - 12 pups in one gestation period.

Before discussing the development of these pups in more detail, let's review the terms used to identify the developmental stages. The most common staging system begins at embryonic day zero, or E0, on the day of successful copulation. After that, each stage is defined by the number of days since fertilization right up through the day of birth when the numbering restarts at postnatal day zero, or P0.

Since developmental timing can vary slightly even between embryos of the same litter, alternative approaches based on morphology rather than time post fertilization, like Theiler staging, can also be used.

Next, let's take a closer look at the morphological changes that occur during those first few weeks of mouse development.

After fertilization of the oocyte, the embryo starts its life slowly, completing only 4 rounds of cell division in its first 3 days. However, by E4 these cells have multiplied and reorganized to form a compacted, hollow ball of cells known as the "blastocyst." At this stage, the cells that will eventually give rise to the embryo itself are all found within a cluster of stem cells known as the inner cell mass, or ICM. The remaining cells, known as the trophoblast cells, will become part of the placenta that provides oxygen and nutrition to the embryo.

After this point, rodent development gets a little twisted. In most mammals, the cells that give rise to the embryo form a disc-like structure. In contrast, the mouse embryo has a cup-shaped configuration. Cells on the outside of this cup form a cell layer known as the endoderm, which eventually gives rise to deep tissues such as the lining of the digestive tract. Confusingly, the cells on the internal surface of the cup represent the ectoderm, which forms more superficial tissues, like hair and skin.

This inverted layout persists until about embryonic day 8, when the embryo quite literally turns itself around. By this point, a few other recognizable structures have developed, including the somites, which give rise to tissues like the skeletal muscle; and the limb buds, which will form the fore and hind limbs.

Things move pretty quickly from here, with the development of major organ systems, such as the lungs and digestive tract, well under way by embryonic day 12. Remarkably, the embryos are ready to survive outside the mother after only 19 -21 days of gestation.

Now that you have a feel for how development proceeds in utero, let's talk about what happens after mice give birth. The newborn mice, or pups, are tiny, hairless, and blind.

For the first few weeks of life, the pups can receive nourishment from any available lactating female. Then, about three weeks after birth, they are ready for weaning, meaning it's time to move out of Mom's place into a cage of their own!

In order to control future breeding, you'll need to separate the males and females at this point. To identify the sexes, examine the distance between the anus and external genitalia. In females, this distance will be shorter than in males.

To prepare the pups' new home, line a cage with a layer of bedding. Since they're still getting used to the new digs, add some food pellets softened with water or a dish of wet food to the bottom of the cage in addition to providing water.

Mice become sexually mature within a few weeks of weaning, with their peak breeding usually falling between 2 and 9 months of age.

So how do we use this information to start a breeding colony? First, it's important to remember that a mouse's behavior is significantly impacted by its circadian rhythm; since they're nocturnal, your mice will breed at night.

Pheromones also play a big part in mouse behavior, so it's helpful to "introduce" potential mates to let them get to know each other. To maximize the number of pups born during a breeding cycle, combine one male mouse with up to 4 females.

To precisely time embryo development, return in the morning to check each female mouse for a vaginal mucus plug, which is deposited by the male during copulation. Mice ovulate every 4 - 5 days; so if you don't see a plug right away, keep the mice together for another chance later that week. Once you've determined that some of the females are pregnant, remove the male mouse from the cage, as they can be a threat to the newborn pups.

In order to study the complex processes controlling the development of mammalian embryos, scientists have developed some very cool techniques. Let's take a look at some examples.

To start, fate mapping is an approach in which cells are marked and tracked in vivo to determine how they contribute to specific structures in developing and adult tissue.

Here, the expression of a fluorescent protein is turned on in a small population of cells to track the contribution of cells expressing the protein to fetal and adult brain tissue.

In order to test the role of a specific gene in development, it is helpful to examine the outcome of its overexpression. In a technique called in utero electroporation, DNA is delivered to the embryo by microinjection and then driven into cells by applying electric current across the tissue. The result is the induction of gene expression in specific cells, as demonstrated by the red fluorescent protein expression in the central nervous system of this embryo.

More permanent changes to gene expression come in the form of knockout mice, in which a portion of a gene is removed. To generate these mice, stem cells are isolated from early embryos and subjected to genome modification. The modified cells are transplanted into a blastocyst, which is then implanted into a female for gestation. The resulting pup will be a "chimera" composed of both normal and knockdown cells, and can be bred to generate homozygous knockout mice.

You've just watched JoVE's overview of mouse reproduction and development. In this video we covered mouse reproduction, prenatal and postnatal development, and how to breed mice. We also discussed some exciting applications for studying mouse development in the lab. Thanks for watching!