



Visual guide for identification of estrus cycle (The Jackson Laboratory)

Improvement by Timed Mating

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Random mating

A complete estrous cycle in mouse spans 4-5 days. Each cycle is composed of metestrus, diestrus, proestrus and estrus. Female in proestrus and estrus are likely to mate upon caging with males and subsequently ovulate and be pregnant. If a random group of females is used for mating, one will expect approximately one third of the females mate. The proportion may decrease when the condition of the animals or the cage environment are suboptimal.

Timed Mating

Timed mating is essential in the production of embryo at defined development stage. Scheduled production of mice at specific age (e.g. Po) by timed mating avoids unwanted births in days not compatible with experimental plan and also reduces the total number of females required for the animal production. It helps as a "reduction" in the principles of the 3Rs.

Estrus identification

Advantage

Identifying females in estrus can largely increase mating efficiency. One may expect 80% of the females in proestrus or estrus mate

after caging with males. It largely reduces the number of males serving as studs. Importantly, one can easily ensure the availability of embryos or fetus in specific days, helping researchers plan experiment ahead easily. For example, a protocol may need a minimal number of new born pups to establish primary cell cultures in a single day.

Methods

Vaginal opening inspection

Appearance of the vaginal opening changes with estrus cycle. Variation is expected between different mouse strains.

- **Metestrus:** small vaginal opening but not closed; the surrounding tissue not swollen; pale and white debris may be apparent.
- **Diestrus:** small vaginal opening and closed; the surrounding tissue not swollen.
- **Proestrus:** wide and moist vaginal opening; the surrounding tissue swollen; pink and wrinkled along the dorso-ventral edges.
- **Estrus:** wide but less moist vaginal opening; the surrounding tissue swollen but less pink.

Vaginal Cytology

Cell smear is prepared from a vaginal swab. Estrous stages are characterized by cell compositions (Biol.Reprod.1984.31:446-53.).

- **Metestrus:** presence of cornified epithelial cells, polymorphonuclear leukocytes and some nucleated epithelial cells.
- **Diestrus:** presence of polymorphonuclear leukocytes. In late diestrus, a few epithelial cells are also found.
- **Proestrus:** predominately nucleated epithelial cells with some cornified epithelial

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cells. In early proestrus, some leukocytes are found.

- **Estrus:** predominately cornified epithelial cells.

Mating

Females at proestrus or estrus are placed in male cages at a female:male ratio 1:1 or 2:1. A male usually gives one productive mating in one night. If the number of males is the limited resource, the optimal female:male ratio is 2:1. On the contrary, if the number of females is the limited resource, the optimal female:male ratio is 1:1.

Mating is confirmed by the presence of vaginal plug in early morning. One may choose to separate the female from the male. Caution should be taken not to put the mated females together with another male because exposing the pregnant females to a stranger male may induce termination of pregnancy.

Embryo/infant stages

Ovulation presumably occurs in the middle of the dark period, i.e. 12:00 midnight, and being defined as day post coitum 0 (dpc 0). The noon following the formation of vaginal plugs are therefore defined as dpc 0.5. Embryos are staged in .5 since experiments are assumed to be performed around noon of the day. Birth of pups is predicted to dpc 19.5 or 20.5 depending on the strains in use, and the day of birth is counted as postnatal day 0 (Po).