Video Article

Harvesting Sperm and Artificial Insemination of Mice

Amanda R. Duselis[1], Paul B. Vrana[1]

[1]Dept. of Biological Chemistry, University of California, Irvine (UCI)

Correspondence to: Amanda R. Duselis at aduselis@uci.edu, Paul B. Vrana at pvrana@uci.edu

URL: https://www.jove.com/video/184
DOI: doi:10.3791/184

Keywords: Developmental Biology, Issue 3, sperm, mouse, artificial insemination, dissection

Date Published: 4/28/2007

Citation: Duselis, A.R., Vrana, P.B. Harvesting Sperm and Artificial Insemination of Mice. *J. Vis. Exp.* (3), e184, doi:10.3791/184 (2007).

Abstract

Rodents of the genus Peromyscus (deer mice) are the most prevalent native North American mammals. Peromyscus species are used in a wide range of research including toxicology, epidemiology, ecology, behavioral, and genetic studies. Here they provide a useful model for demonstrations of artificial insemination.

Methods similar to those displayed here have previously been used in several deer mouse studies, yet no detailed protocol has been published. Here we demonstrate the basic method of artificial insemination. This method entails extracting the testes from the rodent, then isolating the sperm from the epididymis and vas deferens. The mature sperm, now in a milk mixture, are placed in the female's reproductive tract at the time of ovulation. Fertilization is counted as day 0 for timing of embryo development. Embryos can then be retrieved at the desired time-point and manipulated.

Artificial insemination can be used in a variety of rodent species where exact embryo timing is crucial or hard to obtain. This technique is vital for species or strains (including most Peromyscus) which may not mate immediately and/or where mating is hard to assess. In addition, artificial insemination provides exact timing for embryo development either in mapping developmental progress and/or transgenic work. Reduced numbers of animals can be used since fertilization is guaranteed. This method has been vital to furthering the Peromyscus system, and will hopefully benefit others as well.

Video Link

The video component of this article can be found at https://www.jove.com/video/184/

Protocol

Artificial Insemination

Artificial insemination is used instead of natural matings to increase the chances that all the females have will become pregnant. 2 male was used for every 1 superovulated females or a female known to be in estrous. The caudae epididymides from mature male mice were placed in a 9% milk suspension and sliced open with 18 gauge needles. The sperm was allowed to swim freely for a few minutes and then the epididymides were taken from the mixture. The suspension was evaluated with a microscope for confluence and motile sperm. A blunt 22 gauge 1½ inch long needle was used to administer the sperm. A 120° bend was placed about 3/4th the way down the needle. The needle was inserted into the vagina up to the bend and 0.025 - 0.05mls injected. If an assistant is used to hold the female in the appropriate position, the use of anesthetics is not needed.

Preparation

1. Use 4 males for 6 females
2. Euthanize males at the vivarium
3. Prepare about 5 ml of 9% milk from powdered milk
4. Put 1.5 ml in small Petri dish
5. Cut out the epididymus and vas deferens
6. Put in 1.5ml of 9% milk
7. Using two 18 gauge needles, and using long cutting motion with needles, slice open the epididymus and vas deferens to release the sperm
8. Take out left over tissue and check for sperm under microscope

Injection

1. Use blunt needle at 120° angle
2. Hold mouse in hand and inject mixture. If uncomfortable with one person holding and injecting, two people can be used. No anesthetic should be used, as on one knows how this might influence the procedure.

3. Use 0.05ml of sperm/milk mix per female

Recipient females

1. Do vaginal smear on recipient females (by this time the recipient females should be in proestrus/estrus)
2. Females that have not reached estrus by Day 5 should probably not used

Acknowledgements

I would like to thank Mike Dewey from the Peromyscus Genetic Stock Center for his valuable insight, patience, and knowledge on rodent reproduction.

