Benefits of timed mating over blind mating of mice to get timed embryos

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Abstract

Mus Musculus has provided scientists a well-adapted animal for the developmental studies. There is one on one co-relation between higher mammalian genes to mice genes hence making them superior model to study expression and translation research. Albino strain of house mice were used in this study with comparison of random or blind matings in mice and timed pregnant mice. The working behind blind matings is minimal and maintenance time and required force was also minimal as compared to timed matings. The results of comparisons of weight gain with pregnancies was similar in two groups where some un-pregnant mice also showed average or above average weight gain with time. The accurate embryonic stages requirement for the two experiments were e13.5, e14.5 and e15.5 (peak of neurogenesis in mice). The timed matings showed to be superior regarding total pregnancies and having accurate stages and is recommended to be used in order to save trouble of not getting what you require and more sacrifice of mice then is needed.

Introduction

The scientific name of house mice is *Mus Musculus* and it belongs to kingdom animalia, phylum chordate, class mammalian and order rodenta. Mice are preferred in molecular research because they show great similarity with human with respect to genetic, physiological and anatomical properties (Bryda 2013). To investigate human health almost 59% animals used in biomedical research are mice (Taylor and Alvarez 2019). Short life cycle, short gestation period and high breeding efficiency make it excellent as a model organism in laboratory(Merlo, Altruda et al. 2012). More over mice are small, easy to handle and can be transported easily. They are used to study human diseases and human genetics because they are phylogenetically related to human (Perlman 2016). Gestation period of mice is between 18 to 22 days (Murray, Morgan et al. 2010). To understand developmental biology it is necessary to carry out accurate staging of embryos that help to compare embryonic development of mice with same or different species or group of animals (Wong, van Eede et al. 2015). Almost 95% of the genes of human and mice are similar so it is excellent model to study transcriptional analysis (Simon 3, 5 et al. 2002). The estrous cycle of female mice consists of four stages namely proestrous, estrous, metestrous

and diestrous (Byers, Wiles et al. 2012). There are various methods to evaluate the estrous cycle of mice that include visual observation (Auta and Hassan 2016), vaginal cytology (McLean, Valenzuela et al. 2012), histology of reproductive organs (Byers, Wiles et al. 2012), vaginal wall impedance (Rodriguez, Araki et al. 1997) and biochemistry of urine (Singletary, Kirsch et al. 2005). During the estrous stage of the estrous cycle female mice ovulate and breed with male mice (Bertolin and Murphy 2014). For investigating genetics of microcephaly and other developmental disorders mice is most suitable model (Moon 2006).

Material and Method:

Housing of animals

We set up two experiments to get timed embryos at different embryonic days (from e13.5-e18.5) In first experiment we took 10 females mice in a separate cage and housed 5 male mice each in a separate cage to increase their sperm level, fertility and also to avoid aggressive behavior (Zidar, Weber et al. 2019). We used wooden cages that had proper fresh water supply. The pelleted food was given which was made up of ground ingredients. We used fine wooden scrap for bedding. The cages were placed in an air-conditioned room. In second experiment we took 8 female mice and 4 male mice under same conditions.

Experiment 1: Random or blind mating

1. Initially female mice were labeled by using green and black permanent makers. One, two, three and four black bands on the tail of mice represent mice number 1, 2, 3 and 4 respectively while full black tail show mice number 5. Similarly one, two, three and four green bands represent mice number 6, 7, 8 and 9 respectively. Further full green tail shows mice number 10.
2. Body weight of all female mice was measured in the start of the experiment. Then all female mice were shifted male cages in this way that each male cage has two female mice. Vaginal plug was checked in next day early in the morning. The female mice in which vaginal plugs were observed separated from male and housed in a separate cage.
3. Weight of all female mice was recorded on daily basis.
4. At the desired stage the mice were dissected to collect the embryos.

Experiment 2: Timed mating

1. Method of labeling mice was used as previous. Weight of each female mice was also recorded on daily basis.
2. To evaluate estrous stage of mice we use vaginal cytology protocol (Byers, Wiles et al. 2012).
3. Female mice that were in estrous and pro-estrous stage were housed with male mice in the evening.
4. On the next day, in the morning, the female mice were separated from male mice.
5. The remaining female mice were house with male mice when they reached estrous or pro-estrous stage.

Dissection of mice:

On the desired embryonic day the mice were dissected to collect embryos. We preserved embryos in RNA latter, 4%PFA (paraformaldehyde), formalin and some embryos were snap freezed. We did this for further studies

Results

- Weight gain was observed in each mice, though it did not reflected true pregnancy (Table 1).

Mice No.	Initial weight (g)		Final weight before dissection (g)		Net weight gain (g)	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
1	34	22	38	28	4±1	6±1
2	32	14	36	20	4±1	6±1
3	32	26	36	30	4±1	4±1
4	34	26	42	38	8±1	12±1
5	32	14	38	20	6±1	6±1
6	26	26	34	30	8±1	4±1
7	Paralyzed in both experiments					
8	26	28	34	40	8±1	12±1
9	24		32		8±1	
10	26		36		10±1	

Table1: Correlation of mice with weight during the two experiments

The vaginal plug was observed once but it lead to false pregnancy. In random mating we got 4 pregnant mice.

Blind VS Timed matings in mice

Blind matings 10 female with 5 stud males. Experiment took place from 16-05-22 to 20-06-22 (Table 2).

MICE No.	Pregnancy induced after blind mating	Stages in days	Adult tissues collected	Embryonic brain collected	Rest of the embryos collected
1	X	NA	√	NA	NA
2	X	NA	X	NA	NA
3	X	NA	X	NA	NA

4	√	E 6.5	√	√ (Whole embryos)	
5	√	E 12.5	√	√	√
6	√	E 13.5	√	√	√
7	Died	NA	X	NA	NA
8	X	NA	√	NA	NA
9	√	E 18.5	√	√	√
10	Died	NA	X	NA	NA

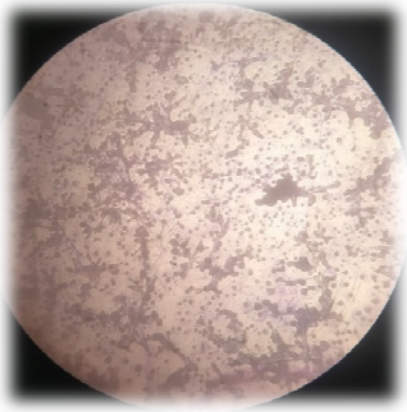
Table 2: Random or blind matings and the success in pregnancies and collection of samples

Timed matings were set on 24-03-23 and finished in 10-05-23. Total of 8 female mice were mated with 4 stud male mice (Table 3).

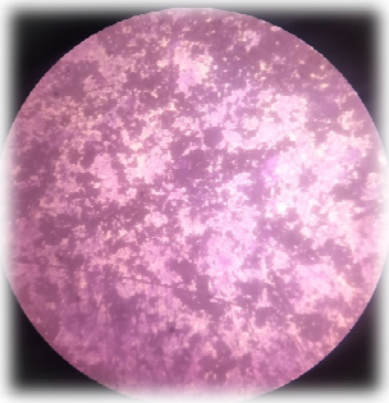
MICE No.	Pregnancy induced after blind mating	Stages in days	Adult tissues collected	Embryonic brain collected	Rest of the embryo collected
1	√	E 14.5	√	√	√
2	√	E 7.5	√	√ (Whole embryos)	
3	√	E 13.5	√	√	√
4	√	E 14.5	√	√	√
5	√	E 15.5	√	√	√
6	√	E 15.5	√	√	√
7	Died	NA	X	X	X
8	√	E 13.5	√	√	√

Table 3: Timed pregnant mice results showing more pregnancies and accurate embryonic stages

- Vaginal cytology of timed pregnant mice. The mice which were in the estrous and pro-estrous phase give accurate stage of embryo (Figure 1).



Pro-estrus phase



estrous phase

Figure 1: The stages visible after crystal violet dye staining vaginal smear which are superior to setup matings at in mice estrous cycle

- Embryonic day was identified by characteristic feature of embryos. Like the embryo of 13.5 stage has following. The foot plate is anterior and posterior distal borders are sectioned now. Hand plate has more cleared sectioned than foot plate. In hind limb only ankle region is identifiable while in forelimb elbow and wrist are distinguishable. Furthermore pinna and whiskers are appearing.

Figure 2: An embryo at e13.5 day

- At 14.5 embryonic stage. The separation of individual digits in the anterior footplate is a unique feature of this stage. Although there isn't yet any discernible separation between the growing toes, the rear footplate exhibits significant indentations.

Figure 3: Three mice embryos recovered at e14.5 stage in mice development

- At embryonic stage 15.5 Complete interdigital indentation is present on the hand and foot plates, and the vanishing of the webbing improves the delineation of each individual digit. The elbow and knee are obviously bent. Hair follicles can be found in the cephalic region, but not along the margin of the vibrissae. The anterior area of the back is gray, and the curvature of the back is markedly different from the currently straight post-cranial vertebral axis.

Figure 4: Two embryos at e15.5 day in mouse staging according to Theiler staging

Discussion:

In the series of experiment done in this study we got contradicting results showing no/very little dependency on weight gain when talking about pregnancies. Average weight gain in random or blind matings in all females with time was 4.5+- 1g regardless of pregnancy. However in the pregnant mice which were 4 out of 10 females average weight was 7.5+_1g. Which was a bit of difficult to judge correct stage of pregnancy as we got 6+-1g weight gain in the unpregnant mice only. We mated the mice everyday and separated in the morning. From mice behaviour and other features we separated females which looked pregnant. The true judgement numbers regarding pregnant to unpregnant mice was 4 and 5 respectively out of total 10 mice. Which is not very significant number. Moreover we were only sure of staging after dissecting out embryos and didn't always find the required range. This experiment was however not very lengthy and took four weeks to complete. The second experiment we ran was timed matings in mice. Total average weight gain in mice regardless of pregnancy was 6+-1g. The pregnant females gained average weight 7.3+-1g and unpregnant mice got average weight gain of around 5.5+-1. The values of weight gains in both experiments were almost comparable but just on the criterion of weight it is hard to judge the pregnancy and more so the exact stage we are looking for as was our requirement in our study. The time taken to finish the second experiment was however lengthy and took almost two months and required more housekeeping of mice and care for the animal requirements.

The second criterion was total number of pregnancies in both experiment in random or blind mating only 4 out of 10 females were found pregnant after dissection. In timed matings on the other hand 7 out of 8 mice got pregnant and apart from one mice all six gave the accurate embryonic stages which were proven by the criterion of the Theilerr mice staging. However we had only small number of animals available for these experiment as we don't have a facility of animal house in our campus. We had to commute to different campus and used their facility under their approved animal use ethics and we respect it.

Conclusions:

The timed matings gave us more pregnant mice at desired stages than mice with blind matings. As previously suggested we confirm same results that although time taken for the whole experiment was more with timed matings and more afforts and housekeeping was required to get the desired results as can be seen from the dates of the experiments the desired outcome was statistically significant than blind matings.

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References:

1. Zidar, J., Weber, E. M., Ewaldsson, B., Tjäder, S., Lilja, J., Mount, J., . . . Törnqvist, E. J. A. (2019). Group and single housing of male mice: collected experiences from research facilities in Sweden. *9*(12), 1010.

2. Auta, T. and A. Hassan (2016). "Alteration in oestrus cycle and implantation in Mus musculus administered aqueous wood ash extract of Azadirachta indica (neem)." Asian Pacific Journal of Reproduction **5**(3): 188-192.

3. Bertolin, K. and B. D. Murphy (2014). Reproductive tract changes during the mouse estrous cycle. The guide to investigation of mouse pregnancy, Elsevier**:** 85-94.
4. Bryda, E. C. (2013). "The Mighty Mouse: the impact of rodents on advances in biomedical research." Missouri medicine **110**(3): 207.
5. Byers, S. L., M. V. Wiles, S. L. Dunn and R. A. Taft (2012). "Mouse estrous cycle identification tool and images." PloS one **7**(4): e35538.
6. Byers, S. L., M. V. Wiles, S. L. Dunn and R. A. J. P. o. Taft (2012). "Mouse estrous cycle identification tool and images." **7**(4): e35538.
7. McLean, A. C., N. Valenzuela, S. Fai and S. A. Bennett (2012). "Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification." JoVE (Journal of Visualized Experiments)(67): e4389.
8. Merlo, G. R., F. Altruda and V. Poli (2012). "Mice as experimental organisms."
9. Moon, A. M. (2006). "Mouse models for investigating the developmental basis of human birth defects." Pediatric research **59**(6): 749-755.
10. Murray, S. A., J. L. Morgan, C. Kane, Y. Sharma, C. S. Heffner, J. Lake and L. R. Donahue (2010). "Mouse gestation length is genetically determined." PloS one **5**(8): e12418.
11. Perlman, R. L. (2016). "Mouse models of human disease: an evolutionary perspective." Evolution, medicine, and public health **2016**(1): 170-176.
12. Rodriguez, I., K. Araki, K. Khatib, J.-C. Martinou and P. Vassalli (1997). "Mouse vaginal opening is an apoptosis-dependent process which can be prevented by the overexpression of Bcl2." Developmental biology **184**(1): 115-121.
13. Simon 3, E. B. I. B. E. G. N. K. A. M. E. R. A. G. S. G. S. A. U.-V. A. W., R. G. i. B. I. A. J. F. G. R. P. G. 5, B. A. P. 6, N. C. f. B. I. A. R. C. D. M. H. W. M. D. R. S. V. 7, D. o. M. A. M. P. L. 8, D. o. M. G. A. S. E. D. E. T. R. A. U. C. 9, C. f. B. Science and E. B. R. D. M. F. T. S. H. A. H. F. K. D. K. W. J. R. K. M. S. Matthias (2002). "Initial sequencing and comparative analysis of the mouse genome." Nature **420**(6915): 520-562.
14. Singletary, S. J., A. J. Kirsch, J. Watson, B. O. Karim, D. L. Huso, P. D. Hurn and S. J. Murphy (2005). "Lack of correlation of vaginal impedance measurements with hormone levels in the rat." Journal of the American Association for Laboratory Animal Science **44**(6): 37-42.
15. Taylor, K. and L. R. Alvarez (2019). "An estimate of the number of animals used for scientific purposes worldwide in 2015." Alternatives to Laboratory Animals **47**(5-6): 196-213.
16. Wong, M. D., M. C. van Eede, S. Spring, S. Jevtic, J. C. Boughner, J. P. Lerch and R. M. Henkelman (2015). "4D atlas of the mouse embryo for precise morphological staging." Development **142**(20): 3583-3591.

17. Zidar, J., E. M. Weber, B. Ewaldsson, S. Tjäder, J. Lilja, J. Mount, C. I. Svensson, E. Svensk, E. Udén and E. J. A. Törnqvist (2019). "Group and single housing of male mice: collected experiences from research facilities in Sweden." **9**(12): 1010.