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Breeding performance with special reference to pre-weaning mortality of C57BL/6 and B6D2F1 hybrid strain of mice maintained at ACTREC Animal Facility

Rahul Thorat[1] and Arvind Ingle[2]

[1, 2] Laboratory Animal Facility, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai, 410 210, MS.

[2] Homi Bhaha National Institute, Mumbai.

Corresponding author:
Prof. Arvind Ingle
Laboratory Animal Facility, ACTREC, Tata Memorial Centre,
Sector 22, Kharghar, Navi Mumbai, 410 210, MS.

Abstract

Successful breeding programme of laboratory animals is one of a key factor in any facility operations. In-house breeding of laboratory animal species depends on using correct breeding method, number of animals requested by the researchers, litter size, birth weight, perinatal mortality, weaning percentage, female:male ratio, parity interval, fostering, postpartum heat, non-productive breeders, enrichment programme etc. To fulfil the animal demands for in vivo research, these points play significant role, especially perinatal mortality. Perinatal mortality is strain dependent and therefore facility must be aware about the percentage of pups mortality to avoid further miscalculation while setting up the breeding program. In order to get the first hand information about strain wise perinatal mortality, historical control data or baseline data should be generated which play an important role in successful breeding programme and timely meeting the target of providing experimental animals. Aim of the present study was to generate baseline control values of reproductive efficiency parameters of commonly used mice strains at ACTREC Animal Facility. The existing in-house data was compiled for the period of July to December 2019. Perinatal mortality varied between the primiparous and multiparous females. In total, data of 4247 pups obtained from 39 inbred primiparous, 93 outbred primiparous, 159 inbred multiparous and 466 outbred multiparous females. The average number of pups delivered by inbred primiparous and multiparous females was recorded as 5.13 and 5.85, respectively. There was no significant difference between the female:male ratio between primiparous and multiparous females of C57BL/6 inbred strain. Weaning of pups was done on day 21. Percentages of weaning of pups from primiparous and multiparous inbred strain were recorded as 87 and 92.37%, respectively. Perinatal mortality in inbred primiparous female was 13% while in inbred multiparous was 7.63%. It is observed that perinatal mortality in inbred primiparous pups is higher than inbred multiparous females. In case of outbred stock of C57BL/6 mice, we observed average litter born of 4.96 and 5.70 for primiparous and multiparous mothers, respectively. Weaning percentage of 83.73% and 93.56% was recorded in outbred primiparous and multiparous mothers, respectively. Perinatal mortality in pups obtained from outbred primiparous and multiparous mothers was 15.84% and 6.44% respectively. The data indicated that perinatal mortality is significantly high in the pups obtained from primiparous females than multiparous females of both the breeding methods. We compared the data of C57BL/6 mice with robust strain, B6D2F1. The overall results can be used as baseline data for our C57BL/6 and B6D2F1 strains by the ACTREC scientists as well as colony managers from other facilities.

Key words: Breeding performance, pre-weaning mortality, C57BL/6 and B6D2F1 mice**.**

INTRODUCTION

It is well known now that laboratory mouse model is well accepted *in vivo* model system. Biomedical research without the use of laboratory animals is inevitable. With the advancement in gene-editing technology, thousands of new strains are being quickly produced by using various well established methods like CRISPR/Cas9 technology. Genetically Engineered Mouse Models (GEMM) mimics several life threatening human diseases. GEMM is an excellent model system to extrapolate the result findings into clinical research. Majority of GEMM are generated after backcrossing onto inbred backgrounds such as C57BL/6 strain of mice (Sztein *et al*., 2000). Among the articles surveyed by Labome (https://www.labome.com/method/Laboratory-Mice-and-Rats.html#ref1), a significant percentage cites the C57BL/6 mouse strain. There were 19807 citations in Pubmed database till the year 2018. This is the first strain whose genome was fully sequenced in the year 2002 soon after the human genome was sequenced. (https://www.labome.com/method/Laboratory-Mice-and-Rats.html#ref1).

It is likely that the C57BL/6 strain will continue to be the preferred strain due to the fact that its genome has been sequenced through the concerted effort of gene functional analysis by International Mouse Phenotyping Consortium (IMPC). B6 (as is an abbreviation of C57BL/6) mouse strain is mainly used to address the research questions in the area of immunological studies, basic cancer research, nutritional studies, and behavioural studies (Song *et al*., 2017, Bryant C. 2011). Considering the importance of B6 strain in wider research area, Animal Facilities should critically design its breeding programme. Mouse breeding is a most critical part of animal facility operations. Investigators solely depend upon Animal Facility to issue animals for experimentation. To make breeding programme successful, one of the important factor is creating the in-house baseline/ historical control data of available strains which includes body weight, feed consumption, clinico-haematology, and breeding performance data etc. The in-house baseline data or historical control data will give important information about breeding efficiency of the strain in terms of litter size, birth weight, incidence of stillborn pups, perinatal weaning mortality and production index of breeders. Perinatal mortality means mortality of pups noticed before weaning, which is calculated by subtracting total number of animals weaned from total number of pups delivered.

Pre-weaning mortality amongst the litters is influential parameter to study breeding performance of a strain. Pup mortality leads to an increased number of mice required for breeding purpose to meet the need of the investigators. This subsequently adds cost burden on Animal Facility to produce more number of animals, additional manpower besides increased space on the racks. Researchers entirely depends upon animal facility to conduct *in vivo* research. Ultimately, the responsibility falls on the animal facility to fulfil the demands of experimental animals. Animals are bred by inbreeding, outbreeding, random breeding or hybrid systems of mating. Beside the type of animal required by the researchers, the choice of breeding method depends upon species and strains to be used. There are several factors which affects the successful breeding programme. Perinatal mortality is a major worrisome factor amongst them.

ACTREC Animal Facility maintains various strains of inbred, outbred, F1 hybrid, immuno-compromised as well as GEMM https://actrec.gov.in/cri-research-support-facility-detail/70. The C57BL/6 mouse is the most commonly preferred strain by our investigators for various cancer studies as well as background strain for transfer of desired gene. Females of C57BL/6 are also used to produce the B6D2F1 hybrid mice by mating with the males of DBA/2 strain. Hybrid mice are genetically identical but heterozygous at all loci and are used in several biomedical research.

The aim of the present study is to compare perinatal mortality between C57BL/6 and B6D2F1 hybrid mice of primiparous and multiparous mothers to establish in-house baseline data for reproductive efficiency of these strains.

MATERIALS AND METHODS

Animals

The data of perinatal mortality of pups were taken from B6D2F1 hybrid mice and inbred as well as outbred colonies of C57BL/6 mice. The original stock of C57BL/6NCrl and DBA/2NCrl was procured from the Charles River Laboratories, Wilmington, USA, whereas B6D2F1 mice are F1 hybrid progeny of crosses between inbred C57BL/6NCrl/Cri females with inbred DBA/2NCrl/Cri male mice. These mice were housed in an individually ventilated animal caging system and provided with commercially available imported corncob as bedding material. All the cages were provided with environmental enrichment of sterile wood shavings for nesting purpose. The animals were housed in a controlled environment at 23 ± 2°C with 40-70% relative humidity and a 12-h/12-h dark/light cycle in the Laboratory Animal Facility of ACTREC, Navi Mumbai which is registered with the CPCSEA, New Delhi for breeding and experiments on small laboratory animals. The animals received sterile water *ad libitum* and gamma irradiated animal feed (Altromin make, Cat no. 1314P having 22-23% crude proteins).

Monogamous method of breeding was adopted for all the breeding pairs. For inbreeding, brother/ sister mating for at least 20 consecutive generations was followed. The presented data is for primiparous as well multiparous inbred, outbred and hybrid breeders. For outbreeding, we used unrelated mates.

Reproductive Parameters and data collection

Existing data from in-house breeding records of ACTREC Animal Facility from July to December 2019 was compiled. To know reproductive performance, we recorded the litter size and litter survival till weaning age of 21 days. Pre-weaning pup survival rate was calculated based on the difference between number of pups born at birth and total numbers weaned. Sexing of the pups was done on day 0 and at weaning on 21 days.

Breeding cages were exchanged once a week and were checked daily for litters born and mortality in the litters. Special care was taken with minimal gentle handling to mother and pups. While checking of the cages, litter born and dead pups were recorded on the cage cards and subsequently in the birth register. At 21 days, all the pups were weaned and segregated into male and females cages. The female:male ratio of weaned animals was recorded during weaning. Breeding pairs were allowed to breed till 6th litter. We termed the mothers at 1st delivery as 'primiparous mothers' and from 2nd delivery onwards we called them as 'multiparous mothers'. The same criteria was applied for inbred, outbred and hybrid breeders as well.

Statistical analyses

All data was analyzed using Prism GraphPad software. We have analyzed the data during the period of July to December 2019. All analyses were performed using a one-way analysis of variance (ANOVA) and unpaired t-test for non-normally distributed data.

Results

The presented data is a comparison between the reproductive efficiency of B6 strain and B6D2F1 hybrid strain. In total, data of 4247 pups delivered from primiparous as well as multiparous females of inbred and outbred C57BL/6 breeders was analysed. The data of C57BL/6 mice is compiled from total 757 numbers of primiparous and multiparous females of inbred and outbred mothers. Out of total 757 females, data from 39 and 159 numbers of inbred primiparous and multiparous females were collected, respectively. While in case of outbred C57BL/6 mice, data from 93 primiparous and 466 multiparous numbers of females were used for analysis.

Thirty nine inbred primiparous C57BL/6 mothers delivered total 200 pups with an average of 5.13 pups per females. At day 0, the female:male sex ratio of 51% (102/200) and 49% (98/200) was recorded, respectively. On the other hand, 159 inbred multiparous C57BL/6 mothers delivered 930 pups with an average of 5.85 pups per female. At day 0, female:male sex ratio of 50.32% and 49.68% was recorded, respectively. There was no significant difference noticed between average number of pups delivered and female:male sex ratio of inbred primiparous and multiparous mothers. Strain wise summary related to C57Bl/6 mice is mentioned in Table 1.

On day 21, the percentages of weaning of inbred pups from primiparous and multiparous females was 87% and 92.37%, respectively. At weaning age, female:male sex ratio of primiparous females was 87.25% and 86.73%, whereas 92.31% and 92.42% of female:male ratio was recorded in inbred multiparous females. There was no significant difference in post weaning ratio of female verses males.

The perinatal mortality was calculated by subtracting total number of pups delivered and total number of animals weaned. In case of inbred primiparous mothers, the cumulative perinatal mortality was recorded 13% (26 out of 200 pups) whereas cumulative pre-weaning mortality of inbred multiparous mothers was 7.63%. This data suggests that there is a clear difference between pre-weaning mortality between inbred primiparous and multiparous mothers.

Likewise, we recorded data from outbred female breeders. Out of 757, total of 93 numbers of mothers from outbred primiparous as well as 466 numbers of multiparous females were analysed. The average litter born for outbred primiparous and multiparous mothers was 4.96 and 5.70, respectively. Ninety three primiparous mothers delivered 461 pups whereas 466 multiparous mothers delivered 2656 pups. At day 21, the total numbers of animals weaned from primiparous and multiparous mothers was 386 (83.73%) and 2485 (93.56%), respectively. The cumulative pre-weaning mortality of pups delivered by outbred primiparous and multiparous mothers was 15.84% and 6.44%, respectively.

Perinatal mortality of pups delivered by primiparous inbred as well as outbred mothers was 13% and 15.84%, respectively. On the contrary, pups from multiparous females of inbred and outbred mothers were 7.63% and 6.44%, respectively.

Our data suggests that perinatal mortality is significantly high in primiparous mothers than multiparous mothers of both breeding systems.

F1 hybrid

For comparison, we utilized data of B6D2F1 hybrid mice. For F1 hybrid, the data were collected from 596 numbers of females, which delivered a total 3661 pups. Eighty nine primiparous mothers delivered 531 pups whereas 507 multiparous mothers delivered 3130 pups. Strain wise summary related to B6D2F1 mice is mentioned in Table 2.

The data from primiparous mothers revealed that, cumulative mortality rate of the pups was 3.20% and from multiparous mothers the mortality rate of pups was 4.54%. There was a statistically significant difference of pups mortality in B6D2F1 hybrid strain in the primiarous and multiparous mothers.

DISCUSSION

Parental care is a key factor of survival of the mammalian offsprings. Parenting influences their adult lives mentally and physically. Naturally, both the parents have responsibility of nest building and feeding but because mothers only lactate, the primary responsibility for parenting falls on mothers. While setting up of breeding nucleus, it is commonly presumed that mortality rates among the pups are higher in the first litters, due to inability of primiparous females to care appropriately for their offspring (Nowak *et al*., 2000). In a comparative study, Brown *et al*., (1999) found higher survival in second litter than in the first litters in C57BL/6J and DBA/2J mice strains. There are several factors associated with maternal behaviour to affect pup survival such as maternal responsiveness (Nowak *et al*., 2000; Nowak and Levy F 2010; Stolzenberg *et al*., 2012) and nest building capacity of females (Canali *et al*., 1991). Most studies comparing primiparous and multiparous females focuses on loss of individual pups rather than loss of entire litters.

Maternal neglect is thought to be the primary cause of pup mortality in primiparous mothers (Champagne *et al*., 2003). However, the causes of pup mortality in primiparous mothers is not studied well, and only a handful of papers exist where mortality was systematically studied. Literature revealed, mortality varies greatly between publications from nearly none to 50% in experimental studies (Reeb-Whitaker *et al*., 2001; Inglis *et al*., 2004; Cooper *et al*., 2007; Whitaker *et al*., 2007) compared to 12.6% reported for the same strain (C57BL/6) from a commercial breeder (Mouse Phenome Database 2011). Although there seems to be strain variations in pups mortality, better managemental practices along with the quality diet can provide the solution to the low pups mortality as seen in this study.

Development of specific neural system in maternal care may also need to be studied (Champagne *et al*., 2003). Histological analysis of the mammary glands of primiparous females exhibiting significant pup mortality may reveal whether the glands are well developed or the pups dies because there is no sufficient milk to the pups owing to the underdeveloped glands not producing sufficient milk. More studies in this direction may be required to understand the correlation of hormonal levels, lactation, mammary gland development and pup mortality. In addition, experiments involving the fostering of primiparous delivered pups with experienced mother may also throw the light on the parenting capacity of the primiparous mothers. Finally, it would be interesting to study if it has a genetic basis of having pups mortality trait in some of the strains.

Table 1. Strainwise summary of C57BL/6 mice

C57BL/6 Mice	Number of Females delivered	Total number of pups born	Average number (Mean ± SD) of pups per female	Total number of female pups	Total number of male pups	Preweaning mortality in female pups	Preweaning mortality in male pups	Cumulative preweaning mortality	Total number of weaning of female Pups	Total number of weaning of male Pups	Cumulative weaning percentage
Inbred Primiparous	39	200	5.13 ± 1.32	102/200 (51)	98/200 (49)	13 (12.75)	13 (13.27)	26/200 (13)	89 (87.25)	85 (86.73)	174/200 (87)
Inbred Multiparous	159	930	5.85 ± 1.85	468/930 (50.32)	462/930 (49.68)	36 (7.69)	35 (7.58)	71/930 (7.63)	432 (92.31)	427 (92.42)	859/930 (92.37)
Outbred Primiparous	93	461	4.96 ± 1.65	239/461 (51.84)	222/461 (48.16)	40 (16.74)	33 (14.86)	73/461 (15.84)	196 (82.01)	190 (85.59)	386/461 (83.73)
Outbred Multiparous	466	2656	5.70 ± 1.82	1333/2656 (50.19)	1333/2656 (50.19)	92 (6.90)	79 (5.93)	171/2656 (6.44)	1231 (92.35)	1254 (94.07)	2485/2656 (93.56)

Values in parenthesis indicates the percentage.

Table 2. Strainwise summary of B6D2F1 mice

B6D2F1 Mice	Number of Females delivered	Total number of pups born	Average number (Mean ± SD) of pups per female	Total number of female pups	Total number of male pups	Preweaning mortality in female pups	Preweaning mortality in male pups	Cumulative preweaning mortality	Total number of weaning of female Pups	Total number of weaning of male Pups	Cumulative weaning percentage
B6D2F1 Primiparous	89	531	5.97 ± 1.75	271 (51.04)	260 (48.96)	6 (2.21)	11 (4.23)	17/531 (3.20)	265 (97.79)	249 (95.77)	514/531 (96.80)
B6D2F1 Multiparous	507	3130	6.17 ± 1.96	1607 (51.34)	1523 (48.66)	81 (5.04)	61 (4.01)	142/3130 (4.54)	1526 (94.96)	1462 (95.99)	2988/3130 (95.46)

Values in parenthesis indicates the percentage.

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