# Reproduction in Domestic Animals 48: 292-296

**Influence of Strain and Parity on the Risk of Litter Loss in Laboratory Mice**

EM Weber1,3, B Algers1, H Würbel2, J Hultgren1 and IAS Olsson3

1Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden;

2Division of Animal Welfare, Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland;

3Laboratory Animal Science, IBMC-Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

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Pup mortality is a considerable problem in laboratory mouse breeding and the view that parity influence survival of newborn mice is widespread. Some evidence suggests that maternal behaviour is related to offspring mortality in mice. Parental experience is a factor that can improve maternal behaviour and offspring survival in some mammals. However, few papers report a relationship between parity and pup survival in mice. We investigated the influence of strain and parity on loss of entire litters of C57BL/6 and BALB/c mice using data from a breeding colony. In total 344 C57BL/6 and 146 BALB/c litters were included. We found a considerable mortality rate for both strains: 32% of C57BL/6 litters and 20% for BALB/c litters were lost. There was a significant difference in survival of first litter between strains, with 3.6 times higher odds of mortality in C57BL/6 mice (P=0.0028). Parity or previous parental experience of litter loss did, however, not affect litter loss.

The scientific literature does not provide a clear picture of perinatal mortality in laboratory mice. Very few studies report perinatal mortality and only a handful of papers exist where mortality was systematically studied; this area is thus poorly understood. If perinatal mortality in mice is not recognized and investigated, but instead considered normal when breeding mice, a serious welfare problem might be overlooked.

## Introduction

Perinatal mortality includes death during late pregnancy as well as young dying within the first week after birth, with most deaths taking place during the first couple of days. Rates of perinatal mortality in domestic animals vary greatly with species and management and the phenomenon is relatively well studied in farm animals, where the major causes of death are similar across species: hypothermia, underfeeding, inappropriate maternal behaviour, infections and injuries (Mellor and Stafford 2004). Pup mortality is a considerable problem also in laboratory mouse breeding. The first (and so far only) detailed description of the phenomenon in mice was published nearly 60 years ago (Hauschka 1952), reporting a mortality of more than 30 % until weaning in mice of the A/Ha strain.

In altricial species such as the mouse, infants are dependent on their mother for nutrients and thermoregulation; thus if females fail to provide appropriate care, infants are likely to die from hypothermia or starvation. There is some evidence of maternal behaviour being related to offspring mortality in mice. Poley (1974) found females that had previously lost all or part of a litter to be more reactive to a sound stimulus than females that successfully weaned a litter. The suggestion that mice that are more sensitive to outside disturbances are more likely to lose their offspring is corroborated by the finding that administering an anxiolytic increased offspring survival (Carter et al. 2002). The cage environment in which females and offspring are housed has also been found to affect survival; several studies report reduced mortality when mice are provided nesting material and / or nest boxes (see e.g. Inglis et al. 2004; Tsai et al. 2003; Weber & Olsson 2006). Genotype is also affecting survival and strain differences in mortality have been reported (Hauschka 1952: A/Ha>DBA, C57BL, C58 and C3H; Brown et al. 1999: C57BL/6J >DBA/2J). Further, quantitative trait loci related to offspring survival have been identified in cross-breeding experiments (Peripato et al*.* 2002).

When breeding laboratory mice it is commonly accepted that mortality rates are higher in first litters and inability of primiparous females to care appropriately for their offspring has been reported (e.g. Nowak et al. 2000). Brown et al. (1999) found higher survival in second than in first litters in both C57BL/6J and DBA/2J mice and several factors associated with maternal experience have been reported to affect pup survival, such as maternal responsiveness (Nowak and Levy 2010; Nowak et al.2000;) and nest building (Canali et al. 1991). Most studies comparing primiparous and multiparous females focus on loss of individual pups rather than loss of entire litters.

The scientific literature does not provide a clear picture of perinatal mortality in contemporary research animal facilities. Pup mortality is only occasionally reported in papers where mouse breeding was part of the experimental protocol, and only a handful of papers exist where mortality was systematically studied. When inferring mortality risks from these papers, caution is warranted as they represent different strains and genotypes held under a range of social and physical housing conditions, and timing and methods used to determine mortality vary between studies. Not surprisingly, mortality varies greatly between publications: from nearly none to 50% in experimental studies (Cooper et al. 2007; Inglis et al. 2004; Reeb-Whitaker et al. 2001; Whitaker et al. 2007,) compared to 12.6% reported for the same strain (C57BL/6) from a commercial breeder (Mouse Phenome Database 2011).

The aim of the present study was to study the effect of strain, parity and loss of previous litters on loss of entire litters through the use of existing breeding data for two common mouse strains, C57BL/6 and BALB/c.

## Materials and Methods

***Animals***

Reproductive data was provided from breeding colonies of the inbred strains C57BL/6 and BALB/c mice (derived from breeding pairs bought from Harlan) held at Justus-Liebig-University of Giessen, Germany in 2005-2006. The breeding colonies were maintained through brother/sister mating with siblings from the same litter and mated in pairs. The male was removed 10 days after mating and females kept singly, or with their offspring but without males or other females, during the last days of pregnancy and throughout lactation. The breeding room contained up to 50 breeding pairs housed in conventional open Macrolon type III cages provided with fir tree bedding (Lignocel 3-4, Ssniff bedding), nesting material (soft tissue, Kleenex) and *ad libitum* access to food (altromin 1324, Lage, Germany) and water. The animal room was temperature and humidity controlled (21±1oC and 50±5% respectively) with a 12:12 h day: night cycle (lights on at 0800 h). The animals were specified pathogen-free (SPF) according to FELASA guidelines and regular tests of sentinel animals took place throughout breeding to confirm the SPF status.

In total 538 litter observations were obtained, of which 48 were omitted due to incomplete data. The resulting dataset consisted of 344 litter observations from 111 parental couples in 12 breeding groups in the C57BL/6 strain, and 146 litters bred by 61 parental couples in 7 breeding groups in BALB/c mice, with all mice in a breeding group originating from the same breeding pair. Each parental couple contributed with between 1 and 8 litters (median 3). Loss of single pups was not recorded and thus litter loss refers to whole litter being lost, coded as a binary outcome (0=litter not lost; 1=litter lost).

***Statistical analysis***

The risk of litter loss was modelled using a generalized linear model in the GENMOD procedure of SAS (version 9, SAS Institute Inc., Cary, NC, USA), assuming a binomial distribution and applying a logit link The clustering of litters from the same parental couple was accounted for by the GEE method (Liang and Zeger1986), using a compound symmetry correlation structure.

Fixed-effect predictors were constructed expressing strain (C57BL/6; BALB/c); parity (primiparous; multiparous), and whether or not there was an earlier record of litter loss in the same parental couple (no; yes). The final model contained strain and parity effects, and the interaction between strain and parity. The predictor expressing loss of earlier litters did not contribute significantly to the model and was therefore not included.. Regression coefficients were transformed into odds ratios (OR). Model-based marginal means were calculated to estimate the effect of parity within each strain separately, and transformed into predicted risks.

## Results

The total mortality rate (calculated as loss of entire litters) was 32% for C57BL/6 and 20% for BALB/c (Table 1). There was a statistically significant effect of strain in the first parity, in that primiparous C57BL/6 females were more likely to lose their litters than primiparous BALB/c mice (3.6-fold higher odds; Wald P=0.0028; Fig. 1), but no effect of strain in older females (P=0.45). First parity accounted for 32% of C57BL/6 litters and 46% of BALB/c litters. In both strains, the effect of parity on litter loss was non-significant (P>0.12, not in table).

There was some dependency of two litters within a parental couple (working correlation was estimated to be 0.20).

One hundred and thirty-four (39%) of the C57BL/6 litters were from parental couples with a record of earlier litter death; of these litters, 52 (39%) died. In BALB/c mice, 29 (20%) of the litters were from parental couples with a record of earlier litter death; of these litters, 9 (31%) died. There was no significant effect of earlier litter loss on actual litter loss.

Descriptive statistics is given in Table 1 and the final model of litter loss is presented in Table 2 and Fig. 1.

## Discussion

The present study investigated the effect of strain, parity and loss of earlier litters on loss of entire litters in laboratory mice through an analysis of reproductive data from a breeding colony of C57BL/6 and BALB/c mice. Mortality was considerable for both strains: around one third of C57BL/6 litters and one fifth of BALB/c litters were lost. We found a statistically significant effect of strain in primiparous females, with higher mortality in C57BL/6. We could however not find an effect of parity in any of the two strains and no effect of earlier litter loss.

Our finding that litter loss is higher in primiparous C57BL/6 mice is in accordance with the study of Brown et al. (1999) showing strain differences in first litter of C57BL/6 compared to DBA/2J mice. However, unlike Brown et al., in our study this difference was only present in the first litter, not in overall litter loss. Differences between mouse strains have been reported for a number of physiological and behavioural characteristics (e.g. Crawley et al. 1997; Knight et al. 2007; Sellers et al. 2011; Wei et al. 2011), including maternal behaviour (e.g. Brown et al. 1999; Chourbaji et al. 2011; Shoji and Kato 2006). Potgieter and Wilke (1997) report a variation in mortality between strains from 20.7 % - 69.6 % in the AKR strain to 15.9 % - 22.4 % in C57BL/6. However, very few studies report differences in perinatal mortality and thus this area is poorly investigated.

Strain differences in perinatal pup survival may be due to differences in maternal ability or differences in pup viability, or both. In pigs, the domestic polytocous species in which this question is most studied, it is known that birth order, time to reach the udder and to consume colostrum are important factors for piglet mortality and growth (de Passilé and Rushen 1989; Baxter et al. 2008; Herpin et al. 1996; Herpin et al. 2001; Tuchscherer et al. 2000) although the importance of these factors may be different under different housing conditions (Baxter et al. 2009). To the best of our knowledge, the role of pup viability has not been investigated in mice. However young from altricial species are totally dependent on their mother for survival, thus maternal behaviour is crucial.

Brown et al. (1999) also found significant differences in maternal behaviour between C57BL/6 and DBA/2J mice. C57BL/6 displayed more active maternal behaviour (nest building and pup retrieval) whereas DBA/2J showed more passive maternal behaviour (resting with, crouching over, nursing pups) and they also suggested a delayed initiation of maternal behaviour in C57BL/6. Since the more active behaviour found in C57BL/6 and more passive behaviour pattern found in DBA/2J parallels differences between these strains in learning tasks (C57BL/6 perform better in active and DBA/2J better in passive avoidance tests), Brown el al. (1999) discuss that differences in maternal behaviour may be due to differences in activity levels between strains. Further, Poley (1974) found a relation between litter loss and differences in emotional reactivity in C57BL and BALB. Chourbaji et al. (2011) found an increased attendance inside the nest for C57BL/6N while BALB/c performed significantly more “neglecting” (digging/climbing and pups outside the nest) behaviours and when comparing five inbred strains, Shoji and Kato (2006) found that CBA/Ca, C3H/He, C57BL/6, and DBA/2 engaged more in maternal behaviours compared to BALB/c. Interestingly, both Chourbaji et al. (2011) and Shoji and Kato (2006) report C57BL/6 to be more engaged in maternal behaviours than BALB/c. One could expect that more engagement in maternal behaviour would increase survival, but in this study we found higher survival in BALB/c, we did, however, not observe maternal behaviour and do not know if the animals in this study differ in that aspect.

When housing laboratory mice the cage environment is often barren and do not resemble a natural habitat for a mouse. This might lead to some kind of disruption of maternal behaviour that has consequences for survival of offspring. A few studies have investigated the environmental impact on offspring survival; Potgieter and Wilke (1997) found bedding material to affect reproductive performance and Inglis et al. (2004) found that a super-enriched environment influenced survival, whereas Eskola and Kaliste-Korhonen (1999) found no affect on mortality when comparing two different bedding materials. However, environmental factors would not only affect survival of first litter unless the survival fully depends on maternal experience.

In a review paper Nowak et al. (2000) report that maternal experience could be a factor affecting mortality in several mammalian young. That mortality is higher in primiparous females is a widespread view in laboratory animal breeding and the Jackson Laboratory report that C57BL/6J females frequently lose their first litter (Breeding Strategies for Maintaining Colonies of Laboratory Mice 2007). In the breeding data that we examined we could not find any effect of parity on loss of whole litter in any of the two strains. Brown et al. (1999) on the other hand found higher survival in second versus first litters in both C57BL/6 and DBA/2J mice; however their study differs from ours in terms of how mortality was measured (Brown et al. measured survival of individual pups whereas we measured survival of whole litters) and of size (48 litters in Brown’s study versus 490 in ours). Canali et al. (1991) found that construction of a straw nest improved over time, leading to lower pup mortality in rabbits. However, when investigating time spent with maternal care in mice, König and Markl (1987) found no effect of parity and Margulis et al. (2005) further report that in a number of studies using altricial species, no relationship between parity and litter survival have been found.

When discussing neonatal mortality it is important to distinguish between death of individual pups and loss of an entire litter. Seen as a natural selection mechanism, loss of single pups might be part of the reproductive picture in polytocous animals (Varley 1992), especially if the female produces very large litters. Under some circumstances it might even be adaptive for a female to kill some pups to ensure survival of the rest of the litter; mouse females have been shown to reduce litter size under restricted feeding (Elwood 1991; König 1989). Elwood (1991) also discuss that disturbance of the litter or nest-site might predict a decreased chance of survival and that a strategy in this case might be to cannibalize the litter and remate to give birth to a new litter (Elwood 1991). However, a female invests a large amount of energy in her offspring and under normal circumstances it does not seem adaptive to lose an entire litter and in the present study females had free access to food and water and there were no obvious disturbances to the cage.

It should be stressed that at present, very few studies reporting perinatal mortality in laboratory mice provide information on how pups die. What is generally found when encountering loss of pups in breeding facilities is dead, partly eaten or pup missing in the cage. It might also not always be clear if authors refer to loss of entire litters or loss of single pups and the method should thus be read carefully before interpreting results on litter loss.

***Conclusion***

There is a difference in survival of first litter between C57BL/6 and BALB/c mice where the underlying cause for this difference remains to be found. This study gives no support to the assumption that it is more common for primiparous females to lose their entire litter. If losses of entire litters are not recognized and investigated, but instead considered normal when breeding mice, a serious welfare problem might be overlooked.

## Acknowledgements

The authors wish to thank Dr. Laurence Coutellier and Anne-Christine Friedrich for preparing the dataset and for helpfully answering questions concerning the data. Analysis of data was completed under grant 2006-0876 (Maternal behaviour in sows and laboratory mice) from The Swedish Animal Protection Agency. Data was collected in the course of a project funded by the German Research Foundation (DFG grant WU 494/1-1).

## Conflict of interest statement

The authors declare no competing interests.

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## Figure legends

## Figure 1. Predicted risks of litter loss in laboratory mice, based on data from 490 litters of primiparous or multiparous females of C57BL/6 or BALB/c strains in 2008. Primiparous BALB/c mice have a significantly lower risk than C57BL/6 mice (P=0.0028).Tables

Table 1. Distribution by strain and parity, and litter loss in 490 laboratory mouse litters of C57BL/6 or BALB/c strains in 2008.

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | Parity | No. of litters | No. of litters lost (%) |
| C57BL/6  BALB/c | 1 2 3 4 5 6 7 8  1 2 3 4 5 6 | 111 90 62 36 25 14 5 1  61 45 26 8 4 2 | 39 (35) 27 (30) 16 (26) 10 (28) 11 (44) 4 (29) 3 (60) 0 (0)  8 (13) 12 (27) 6 (23) 2 (25) 1 (25) 0 (0) |

Table 2. Generalized linear model of risk of litter loss in laboratory mice, based on 490 litters of primiparous or multiparous females of C57BL/6 or BALB/c strains in 2008.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Predictor | Category | Coefficient | Standard error | OR | P |
| Intercept  Strain  Parity  Strain by parity Interaction | -  C57BL/6 BALB/c  primiparous multiparous  C57BL/6 and primiparous | -1.22  0.254 -  -0.675 -  1.02 | 0.282  0.334  0.435  0.496 | -  1.20  0.509 -  2.78 | <0.0001  0.45  0.12  0.039 |

## Figure 1

0

0.1

0.2

0.3

0.4

Primiparous

Multiparous

Risk of litter loss

C57BL/6

BALB/c

***Corresponding author***

Elin Weber

Department of Animal Environment and Health

Swedish University of Agricultural Sciences

P.O. Box 234

SE-53223 Skara

Sweden

E-mail: Elin.Weber@slu.se