Pup Mortality in Laboratory Mice

Influence of Maternal Behaviour
and Housing Environment

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Cover: Mouse pup struggling to get back into the nest
(Photo: Elin Weber)

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Pup Mortality in Laboratory Mice. Influence of Maternal Behaviour and Housing Environment

Abstract

Successful mouse breeding is a crucial part of providing animals for research. However, loss of single pups or entire litters after birth is a relatively common problem. Determining how pups die is crucial for the understanding of mortality, but the scientific literature does not provide a clear picture of pup mortality and the reason why pups die is still poorly understood.

The overall aim with this thesis was to investigate the causes of pup mortality in laboratory mice, focusing on maternal behaviour and the effect of housing environment. Specifically the aims were to investigate if litter loss was higher in primiparous females (study 1), if female mice actively killed their pups and if there were any differences in behaviour between females that lost the litter shortly after birth and females that successfully weaned their litters (study 2), and how the conditions for nest building influenced nest building and pup survival (study 3).

In study 1 (paper I), breeding data from mice of the strains C57BL/6 and BALB/c were used. An effect of strain but no effect of parity on litter mortality was found. In study 2, C57BL/6 females were housed in four different treatments with different amounts of nesting material and cage furnishment. Behaviours of females whose litter died were observed in detail from birth of the litter until the litter died (paper II). No evidence that females actively killed their pups was found. In paper III, both females that lost their entire litter shortly after birth and females that successfully weaned their litter were observed from 24h before to 24h after parturition. Litter loss was associated with females showing less nest-building behaviour before parturition, more parturition-related behaviours and more time outside the nest. In the last study (paper IV) females were housed in four treatments with different amount of nesting material and structure present or absent. Females given a larger amount of nesting material built more dome shaped nests of higher quality.

In summary, this thesis does not support the assumption that female mice actively kill their offspring. Pregnant females should be given a large amount of nesting material to enable nest-building behaviour. Further, monitoring females around time for parturition should be considered to detect problematic parturitions.

Keywords: maternal behaviour, postnatal mortality, nest building, nesting material, laboratory mice, breeding, pup survival

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Dedication

To Robert and Piri

and to all the mice out there…

The first step toward change is awareness.
Nathaniel Branden

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Weber, E.M., Algers, B., Würbel, H., Hultgren, J., Olsson, I.A.S. (2013). Influence of Strain and Parity on the Risk of Litter Loss in Laboratory Mice. *Reproduction in Domestic Animals* 48, 292-296.

II Weber, E.M., Algers, B., Hultgren, J., Olsson, I.A.S. (2013). Pup mortality in laboratory mice – infanticide or not? *Acta Veterinaria Scandinavica* 55, 1-8.

III Weber, E.M., Hultgren, J., Algers, B., Olsson, I.A.S. Mortality in laboratory mouse pups – do females that lose their litters behave differently? (Manuscript)

IV Weber, E.M., Hultgren, J., Olsson, I. A. S., Algers, B. Nest quality and pup survival in laboratory mice given different conditions for maternal nest building. (Manuscript)

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1 Introduction

Mice are incredible animals. With their amazing ability to adapt and eat a wide variety of food, they inhabit almost every ecological niche in the world and are the most widespread mammal on the planet. The house mouse (*Mus musculus*) has lived in close association with human populations since civilization first appeared (Silver, 1995). Selectively bred mice are also highly valued as experimental animals and have been used in research for nearly 100 years (Harper, 2010), today constituting the most commonly used vertebrate species in biomedical research. Seven million mice were reported being used in the European Union 2011 (Seventh Report from the Commission to the Council COM(2013) 859 final) and approximately 25 million mice used worldwide (Harper, 2010).

Their short reproductive cycle, short life span, small size and low maintenance costs are characteristics that have contributed to the mice becoming the most popular mammal in research (Baumans, 2004). A crucial part of providing animals for research is successful breeding. Still, in many facilities breeding efficiency is complicated by problems with reproduction, such as pre-weaning pup mortality. Both single pups and the entire litter can be lost, with loss of entire litters having the most substantial influence on breeding efficiency. It is sometimes assumed that it is normal for laboratory mice to lose their first litter due to the mother being inexperienced. Since dead mouse pups are often eaten by their mother, there is further a widespread belief that the female actively kills them. However, the scientific support for these assumptions and the overall understanding of what causes pup loss and how mouse pups die is still insufficient. To increase this understanding, and potentially improve welfare of laboratory mice, it is important to consider the behavioural biology of the wild house mouse (Latham & Mason, 2004), and how their needs might be influenced by the housing conditions provided in the laboratory.

1.1 Free-living house mice

Free-living mice are burrowing animals that are mainly nocturnal (Walker & Nowak, 1999; Sayler & Salmon, 1971). They are very active and agile animals with excellent sense of balance and can run fast up almost any vertical surface or horizontally along small ropes and move hanging upside-down from 6mm hardware mesh with ease. They are excellent jumpers and can squeeze through very small openings (Roll, 2009). Mice are also highly explorative and in the wild they spend a substantial time on seeking a wide variety of food.

Mice form complex social structures (Baumans, 2004) and two common types of populations have been described, commensal and feral (Bronson, 1979). Commensal mice rely on humans for food and shelter and live in territories with stable and plentiful food supply with a population density of up to 10 mice per m^2. Feral populations are less dense (up to 1 mouse/m^2), do not depend on humans and are found in environments with seasonally unstable food supply (Bronson, 1979).

Further, all mice build nests in which they sleep, seek shelter and take care of their offspring. Nests are built in underground burrows or hidden places above ground and are lined with grass, dried plants or other soft materials (Van Oortmerssen, 1971). When giving birth, pairs or groups of females residing within one male’s territory usually form a communal nest and also nurse their pups communally (Manning *et al.*, 1995; Packer *et al.*, 1992; Wilkinson & Baker, 1988). The relatedness of the females and their offspring is probably an important aspect of communal nesting; nest mates who grow up together typically have the same father and females sharing the same nest are often related. Whether her own or the other females’ offspring, it is therefore highly likely that any pup a female nurses will be closely related offspring when communally nesting with a familiar female (König, 1994).

1.2 Housing and management in the laboratory

In the laboratory, mice are typically housed in small plastic transparent cages with wire tops, provided with bedding material and sometimes nesting material. Food from a food hopper in the wire top and water is generally provided *ad libitum*. Caging systems can be either open or individually ventilated and rooms are maintained with controlled dark: light cycle, temperature and humidity. Overall, the housing and husbandry practices in the laboratory have been designed to provide a standardised environment, with main focus on the physical health of the animals, economy and human ergonomics (Baumans, 2010). These conditions generally do not meet the needs of the animals; little consideration has been given to natural behaviour, preventing animals from performing many motivated behaviours and giving them little control over their environment (Olsson & Dahlborn, 2002).

However, despite being bred for hundreds of generations in the laboratory environment, mice still have a strong motivation to perform many of the behaviours seen in their wild ancestors. It has been argued that behaviours essential for survival in the wild will remain highly motivated also in animals in captive environments (Dawkins, 1998; Dawkins, 1990). Nest building is one such behavioural need; both breeding and non-breeding laboratory mice still have a strong motivation to build nests (Olsson & Dahlborn, 2002; Estep *et al.*, 1975). They will work for access to nesting material (Roper, 1976) and when offered a choice, they show a strong preference for access to nesting material (Van de Weerd *et al.*, 1998). When presented with different types of nesting material, mice given more "naturalistic" material built nests of higher quality (Hess *et al.*, 2008). Access to nesting material thus enables mice to perform nest-building behaviour. It also provides shelter and thus a possibility to escape from potential stressors. To a certain degree, nesting material gives mice a chance to control the microclimate in the cage (Gaskill *et al.*, 2011).

The laboratory cage obviously differs from the habitat of the wild mouse ancestors in several aspects also relevant to reproduction. Breeding systems used in the laboratory consists of breeding pairs (one male and one female), trios (one male and two females) or harem groups (one male and several females). In pairs and trios, the male and females are usually kept together to enable postpartum mating. In harem groups, the females are often placed in separate cages when pregnancy is confirmed. If females are housed in groups or individually when giving birth usually depends on the importance of determining which female the pups belong to. In the wild on the other hand, it would probably be rare for a female to raise a litter alone without the presence of the male or other females. Also the weaning process differs. In the laboratory, mouse pups are generally weaned at the age of 21 days, corresponding to the time when the next litter will be born if the female was mated postpartum. However, Bechard and Mason (2010) report that laboratory mouse independence occurs weeks after this age, and this early weaning age might deprive mouse pups of maternal care.

Confined in a laboratory cage, mice have very limited possibilities to adjust their environment. The amount and material of the bedding provided usually limits them to dig more than a few centimetres and thus do not enable creation of burrows. Further, they cannot choose nest site and are restricted to the nesting material provided by the laboratory staff. The nest will thus not be situated in a burrow or a hidden place but instead in a brightly illuminated room. Wallace (1981) found the provision of extensive nesting and burrowing opportunities to be crucial for successful breeding of wild mice in the laboratory. Laboratory mice are typically also kept at a temperature of 20-24˚C which is below their thermoneutral zone (Gaskill *et al.*, 2009). Depending on their ability to create a nest of sufficient quality, they might thus also be exposed to cold stress.

Giving animals the opportunity to perform motivated behaviours, access preferred environments and give them control over their environment are important aspects of welfare of captive animals, and are also required in the European Union according to Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (OJ L 276, 20.10.2010, p.33).

1.3 Reproduction

The reproductive strategy of mice is to produce many large litters, and under favourable conditions, female house mice can give birth to 6-10 young every month. Reproductive performance varies widely between strains (Silver, 1995) but generally, they sexually mature around the age of 6–8 weeks with oestrus cycles that last between 4 and 6 days (Berry, 1970; Bronson *et al.*, 1966). The length of individual cycles varies, and is influenced by season, diet and environment (Baumans, 2004). Fertilization is possible about 10–12 h after ovulation and gestation lasts for 19–21 days. Parturition usually takes place during the night, and is followed by postpartum oestrus with ovulation at 12–18 h after giving birth (Berry, 1970), making it possible for female mice to be pregnant and raise a litter simultaneously.

1.4 Maternal behaviour

In altricial species such as the mouse, maternal behaviour is crucial for the survival of offspring. Since mouse pups have poor thermoregulatory abilities up to 2–3 weeks of age, the construction of a nest before parturition is important for successful rearing of young (Lynch & Possidente Jr, 1978). Maternal nest building in mice starts already around day 4 after mating and the mouse thus differ from other altricial species such as rats and rabbits in that the maternal nest is prepared so early in gestation (Lisk, 1971; Lisk *et al.*, 1969). The nest built by the pregnant female (and sometimes also the male) before parturition differs in shape and structure from sleeping nests and is often referred to as a maternal nest. These nests are 2-3 times the size of a sleeping nest, completely covered and with one or two entrances (Gandelman, 1973b). The pups are thus born in a protected dark, warm chamber. Lisk *et al.* (1969)

reported that nest size continues to increase throughout gestation until 1 day prepartum, and then gradually decreases after parturition, while other authors report a peak in the amount of nest material used around days 12–14 of pregnancy (Broida & Svare, 1982). The hormones oestradiol and progesterone seem to act in synergy to facilitate nest-building behaviour (Lisk, 1971).

During the first 12 days after birth, mouse pups are fully dependent on their mother for nutrition, temperature regulation and to stimulate defecation. During this period the female spends most of her time close to the pups, gathering them in the nest and crouching over them, and she only leave the nest for short bouts (König & Markl, 1987). During the first 5 days after birth, the behaviours spontaneous licking, changing suckling position, and nest building decrease in female mice, and external stimuli from the pups are crucial for maintaining maternal care (Ehret & Bernecker, 1986; Cohen-Salmon *et al.*, 1985). Pups have very limited abilities to move and the mother's ability to retrieve the pups if they fall outside the nest is important for survival: without the insulation from the nest and the mother mouse pups rapidly lose body temperature.

1.5 Pup development and behaviour

Mouse pups are born without hair (except for whiskers), are blind, deaf, have undeveloped motor skills, only weigh approximately 1 g (König & Markl, 1987; Ewer, 1968) and typically huddle to keep warmth. They seem to start hearing by the fourth or fifth day, and by day 6 the body is covered by a thin coat of hair. The eyes open between days 12 and 14 after birth (Fuchs, 1981; Williams & Scott, 1953) and after this they start to become active outside the nest. Except for when the pups are exploring, the eyes are often kept tightly closed until days 15 or 16. At the age of 17 days the hair coat is fully developed and the pups start to eat solid food and the weaning process gradually begins (König & Markl, 1987; Williams & Scott, 1953).

Although they are born with non-functional auditory systems (Porter, 1983), newborn rodents of several species use vocalizations (Elwood & McCauley, 1983). During the first 2–3 weeks postpartum, pups emit a variety of ultrasonic vocalizations when they are isolated from the mother (Branchi *et al.*, 1998). Wriggling calls increase between birth and day 5 postpartum (Ehret & Bernecker, 1986), functioning to maintain maternal behaviour at a high level. Pups have been found to emit wriggling calls regularly during suckling, and always in association with pup movements and ultrasonic sounds can be categorised according to the response triggered in the mother (Ehret & Bernecker, 1986). Factors in the environment can also influence the emission of calls, such as isolation, low temperature and tactile stimulation (Branchi *et al.*, 1998).

1.6 Postnatal mortality

Pup mortality is a considerable problem in many facilities breeding mice. Both individual pups and entire litter can be lost, and they are usually lost the first days after birth (Brown *et al.*, 1999). Losing a small proportion of a litter or losing the whole litter is radically different in reproductive terms for the female. If only single pups are lost she will sustain lactation until the litter is weaned with more resources available for remaining pups than if no pups were lost. If the entire litter is lost she will be able to dedicate all resources to a new pregnancy avoiding competition for resources between pregnancy and lactation which may reduce litter size (McCarthy, 1965). It is difficult to get a good picture of pup mortality in research facilities. Few papers exist where mortality is systematically studied in healthy animals. Studies reporting mortality rates use different strains held under different social and physical housing conditions, and the timing and methods used to determine mortality vary. The reported mortality rates thus vary greatly between publications; from nearly none to 50% in experimental studies (Cooper *et al.*, 2007; Whitaker *et al.*, 2007; Inglis *et al.*, 2004; Reeb-Whitaker *et al.*, 2001), compared to 13% reported for the same strain (C57BL/6) from a commercial breeder (Mouse Phenome Database, Accessed 2011).

The phenomenon of young dying shortly after birth is not unique for laboratory mice. 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 & Stafford, 2004). High numbers of young dying has also been reported in the farmed mink with hypothermia (Malmkvist & Palme, 2008) as well as birth problems and prolonged parturition (Malmkvist *et al.*, 2007) described to be associated with early kit mortality.

1.6.1 Infanticide and cannibalism

Pup mortality in mice is often described in terms of cannibalism or even infanticide, suggesting that the female actively kills her offspring. However, in most instances of mortality, the methodology used does not allow scientists to determine if pups were actively killed or injured or whatever other causes they died from. Cannibalism is defined as the eating of (flesh of) conspecifics (Lawrence *et al.*, 1995), including both killing followed by eating and eating conspecifics already dead (sometimes distinguished as active and passive cannibalism) (McFarland, 2006). Infanticide is defined as the killing of young by conspecifics (McFarland, 2006; McCarthy & vom Saal, 1985).

The phenomenon of infanticide in terms of killing non-related offspring is well known and part of the reproductive strategy of several species. In lions for example, a new male will sometimes kill the cubs present if he takes over the pride (Krebs & Davies, 1993). The explanation for this behaviour is that losing her young will make the female receptive to mating again faster, and this gives the new male an opportunity to mate with her. Mice have been used as model organisms in laboratory studies addressing infanticide from a behavioural ecology perspective (Elwood, 1991). In these studies, males or females with differing sexual experience are exposed to related or unrelated pups and the pups are generally placed in the home cage of the test animal and behaviours measured in so called screening tests (Perrigo *et al.*, 1993; McCarthy & vom Saal, 1986; Gandelman, 1973c; Gandelman, 1973a). Infanticidal tendencies have been reported to differ both within and between inbred laboratory strains (Perrigo *et al.*, 1993) and wild-type mice have been reported to be more likely to exhibit infanticide (Jakubowski & Terkel, 1982; McCarthy, 1965), however screening tests are experimental set ups and not measures taken under normal husbandry conditions.

Female mice have been found to reduce litter size when food is restricted (Elwood, 1991; König, 1989), and Poley (1974) suggested that stress causes females to neglect, kill or eat their young. It should be noted, however, that under normal breeding conditions what is usually found is pups that are partly eaten, or a reduced number of pups, but no evidence of active killing.

In several papers authors refer to cannibalism as the cause of death, *e.g.* “these losses were attributed to cannibalism” (Seamer & Chesterman, 1967), “the majority of deaths (…) due primarily to cannibalism” (Morse *et al.*, 1974), “some of the mice displayed cannibalism toward their newborn pups” (Kang *et al.*, 2004), “cannibalism of newborn mice by their consomic mothers was more frequent than in parental strains” (Gregorova *et al.*, 2008), even in the absence of any information on how cannibalism was defined or observed. Others refer to the loss of pups as infanticide (Shieh *et al.*, 2008; Stahl & Kaneda, 1999) even though there is nothing in their description of how animals were inspected that suggests they could confidently conclude that any active killing took place. In contrast, Macbeth *et al.* (2010) described that pups were whole when found dead in the cage and concluded that females did not appear to attack their young; instead the underlying causes of pup deaths remained unknown.

1.6.2 Genetically modified mice

Today, thousands of different mouse models are used to study the biological functioning of mammalian genes, in 2007 Collins *et al.* reported that 9000 knockout models had been generated. In cases where gene mutations lead to neonatal death, pup deaths are not always a direct consequence of the primary defect, but often caused by physiological problems that arise as secondary effects (Turgeon & Meloche, 2009). Examples of morphological defects leading to neonatal death include shortened jaws and limbs, absence of limbs, lungs, eyes and nose, skeletal defects, craniofacial defects (leading to abnormal suckling), inability to open the jaws (leading to inability to vocalize resulting in rejection by the mother) (reviewed in Turgeon & Meloche, 2009). Poor maternal behaviour has been found in several models and some even show complete inability to rear offspring. Brown *et al.* (1996) found the *fosB* mutant mouse females to neglect their young, and the pups were found scattered around in the cage; the mutant mouse *staggerer* failed in removing the amniotic membrane, leading to pups dying from being choked, and pups that survived died from cold or hunger (Guastavino, 1984). $G\alpha_{q/11}$-deficient females delivered pups normally but did not build nests, gather pups or crouch over them and the pups died scattered in the cage within 48 hours after birth (Wettschureck *et al.*, 2004). Also the mutant *hubb/hubb* (Alston-Mills *et al.*, 1999) and the *Mecp2*-deficient mouse model (Jugloff *et al.*, 2006) are reported being difficult to breed.

1.7 Ethical concerns

In 1876, the first legislation concerning animal experimentation was set in the United Kingdom and for many years this was the only country protecting animals used for scientific purposes by legislation. The first European-wide legislation was established in 1986 with Directive 86/609/EEC. Today, animals used in countries in the European Union are protected under the Directive 2010/63/EU. According to this directive, projects where animals are part of the study must be authorised by the competent authority before the experiment can start. Prior to being approved, projects must be evaluated in an ethical review process, taking into account the ethical considerations of using animals.

Applied animal research ethics is guided by the principles of the 3Rs (Replacement, Reduction and Refinement), established more than 50 years ago by Russell and Burch (van Zupthen, 2001). The aims with the 3Rs are to use methods that *avoid or replace the use of animals* (Replacement), *minimise the number of animals used per experiment* (Reduce) and *minimise suffering and improve animal welfare* (Refinement). In the ethical review process, unless the aim is to produce genetically modified mice or the breeding itself is part of the experiment; breeding of animals is not evaluated since this is not included in the experimental set up. However, since they are laboratory animals, the principles of the 3Rs are still applied.

The work presented in this thesis focus on Reduction and Refinement. With more knowledge about what causes pup mortality in laboratory mice, the number of breeding animals needed to supply experimental animals can potentially be decreased (Reduction). Increased knowledge can also minimise suffering in both female and pups and by investigating the effect of housing environment the overall welfare might also be improved (Refinement).

2 Aims of the thesis

The overall aim with this thesis was to investigate the causes of pup mortality in laboratory mice, with focus on maternal behaviour and the effect of housing environment. Specifically the aims were to investigate:

- If litter loss is higher in primiparous than in multiparous females and if it is more likely for a female that lost a litter to lose another litter (paper I)

- If female mice actively kill their pups (paper II)

- If there are any behavioural differences between females that lose the litter shortly after birth and females that successfully wean their litters (paper III)

- How the conditions for nest building influence maternal nest building and pup survival (paper IV)

3 Materials and methods

This is an overview of the materials and methods used in the four studies included in this thesis. For full descriptions, see paper I-IV. In the first study (paper I), data from a breeding colony of laboratory mice kept at the Justus-Liebig-University of Giessen, Germany were used. The other three studies were experimental. Study 2A and 2B were conducted at the Institute for Molecular and Cell Biology, Porto, Portugal (paper II and III). These studies were carried out between June and September, 2005 (study 2A) and August 2006 and March, 2007 (study 2B). Study 3 was conducted between August 2010 and May 2011 at a large research facility in Sweden (paper IV).

3.1 Animals and housing (study 1-3)

In all studies, mice of the inbred strain C57BL/6 were used since this is the most widely used strain in research and also often used as a background strain when genetically modifying mice. In study 1 breeding data from the inbred strain BALB/c were also utilised, which is another commonly used strain. In study 2B the knockouts Hfe$^{-/-}$ mice and ß2m$^{-/-}$ were included. These were part of the study since the researchers using them reported problems with reproduction and were interested in investigating the effect of housing environment on their reproductive success. Females in all studies were separated from the male before giving birth and housed singly until the litter was weaned.

Animals in study 1 were housed in Makrolon III cages provided with nesting material (housing treatment S). Animals in study 2A were housed in Makrolon II cages without nesting material (B) or in Makrolon III cages with nesting material and furnishment (F; Figure 1). Animals in study 2B were housed in Makrolon II cages with a small amount of nesting material (S) or with twice the amount of nesting material and furnishment (F; Figure 1). In study 3 animals were housed in individually ventilated cages (IVC) type 1291H in one of four treatments: small (S) or large (L) amount of nesting material, and nest structure (S) present or absent (Figure 2). For an overview of strains and housing treatments used in the different studies, see Table 1. Room conditions for all studies were standardised with temperature maintained at 19-23 °C, relative humidity at 40-70% and a 12:12 h light/dark cycle. Animals were given *ad lib* access to food in the food hopper, and autoclaved water. Animals in study 3 were also provided with food on the cage floor.

Figure 1. Housing treatments used in study 2. On top study 2A: left barren, without nesting material (B) and right furnished larger cage, half a tissue paper and furnishment (F). At the bottom study 2B: left standard, 0.5 nestlet (S) and right furnished, 1 nestlet and furnishment (F). (Photo: Elin Weber)

Figure 2. Housing treatments used in study 3. Top left 0.5 nestlet (S), top right 3 nestlets (L). Bottom left 0.5 nestlet and nest structure (SS), bottom right 3 nestlets and nest structure (LS). Food provided on the cage floor. (Photo: Elin Weber)

Table 1. *Overview of strains and housing treatments (cage type, bedding, nesting material and furnishment) included in studies 1-3.*

Study	Number of females	Strain	Cage type (size LxWxH)	Bedding and nesting material	Furnishment	Housing treatment
1	111	C57BL/6	Makrolon III (410×265× 175 mm)	Fir tree, tissue paper	None	S
	61	BALB/c				
2A	10	C57BL/6	Makrolon II (265×205× 140 mm)	Corncob, no nesting material	None	B
	10		Makrolon III (410×265× 175 mm)	Corncob, 1 dl soft bedding, half a tissue paper	Chew block, red PVC nest box, modified cardboard nest box	F
2B	10	C57BL/6	Makrolon II (265×205× 140 mm)	Corncob, 0.5 nestlet	None	S
	11	Hfe$^{-/-}$				
	10	ß2m$^{-/-}$				
	10	C57BL/6		Corncob, 1 nestlet	Chew block, transparent tinted mouse tunnel hanging from the grid, modified cardboard tube nest box	F
	11	Hfe$^{-/-}$				
	10	ß2m$^{-/-}$				
3	14	C57BL/6	IVC 1291H (425×266× 185 mm)	Aspen, 0.5 nestlet	None	S
	14			Aspen, 3 nestlets	None	L
	14			Aspen, 0.5 nestlet	Structure	SS
	15			Aspen, 3 nestlets	Structure	LS
	15			Aspen, 0.5 nestlet	None	C[a] (control)

[a] Control group left undisturbed except for day 2 after birth.

3.2 Data collection

3.2.1 Pup survival (study 1-3)

For study 1, data from existing breeding records were obtained where the numbers of weaned or dead pups were indicated; losses of single pups were not recorded. There is a widespread practice of leaving periparturient females undisturbed around the time for parturition to avoid females killing their young. In study 2, females were therefore left undisturbed and the number of pups in each cage was counted at first cage cleaning after birth (study 2A day 10, study 2B day 4). In study 1 and 2, survival was thus only measured at litter level and litter loss was defined as all pups in a litter dying before weaning at day 21 after birth. However, in several studies using early handling protocols, mouse pups are handled without this procedure leading to pup loss. In study 3, the cages were therefore inspected daily and the mother and pups were also handled from day of birth. Pups were counted on day of birth (day 0) and days 1, 2, 3 and 23 after birth and the loss of both individual pups and entire litters was included in this study. In study 3 dead pups were photographed and it was noted if the pups were partly eaten, injured or intact.

3.2.2 Video recordings (study 2)

The aim of study 2 was to observe females in detail around the time of parturition. Mice are easily disturbed, they are mainly nocturnal and the exact time of parturition is very difficult to determine by only visually inspecting the cages. The females were therefore video recorded in their home cages from approximately 3 days before until 4 days after parturition. Four cages were recorded simultaneously using cameras (Ikegami ICD-47E, B/W CCD, Japan) connected to a time lapse recorder (Panasonic AG-TL750E, Thailand). The recordings were rotated by means of a camera switcher (Sanyo VQC 809-P, Japan) at 30 s intervals. In study 2B, approximately one third of the cages were recorded continuously throughout the recording period with data collected into a computer with a multi camera vigilance system (GV-800/8; GeoVision, Taipei, Taiwan).

3.2.3 Time of birth (study 2 and 3)

To determine day of birth, cages were visually inspected daily from day 18 after mating (study 2) or by removing the cage from the rack and lifting the cage lid (study 3), continuing until the litter was born. In study 2, video recordings were scanned to determine the exact time when parturition began. After detection of pups the film was rewound and played at fast speed forward to find the female in birth position (Ewer, 1968). Time for parturition was defined as the time when the first pup was delivered, or (if the pup was not seen) the first time when the female was seen in birth position. In cages with nesting material it can be difficult to detect the pups and see when the female starts to give birth. If neither the first pup nor birth position was possible to detect, time for parturition was estimated as the midpoint between the last time the female was seen pregnant and the first time a pup was seen or the female was seen non-pregnant.

3.2.4 Behavioural observations (study 2)

In paper II, the aim was to investigate if females actively killed their offspring. Video recordings from females that lost their entire litter before weaning were selected and observed in detail from birth until the entire litter was lost. It is very difficult to observe females in detail when they are housed with nesting material. Therefore, only females from study 2A that were housed without nesting material (n=5) and females from study 2B with a small amount of nesting material (n=5) were selected. The Observer XT 6.1 software (Noldus Information Technology, The Netherlands) was used for scoring behaviours. Both scan sampling and continuous observations of certain time periods were used. A pup was defined as dead when it was lying still and never seen moving again. Behavioural observations started when the first pup was born. First, movements of pups were observed immediately after birth and 1 h postpartum to determine if the pup was born alive or stillborn. Then the female and pups were observed at certain time points, using a predefined flowchart, to detect when each individual pup died. During this scan, only the behaviours “pup still” and “pup moving” were recorded. When “pup still” was observed, the pup was tracked backwards to observe what took place before it stopped moving. To establish time of death, the sequence from when a pup was last seen moving until it was still was observed in detail for all pups dying.

In paper III, the aim was to further investigate the cause of litter loss by comparing the periparturient behaviour of females that successfully weaned their litter, and females whose entire litters were lost. If more than 12 hours of recordings were missing, the females were excluded and in total 64 females from study 2 were used (study 2A n= 17, study 2B n=47). Females were observed on video from 24 hours before until 24 hours after birth and coded by one observer using a predefined ethogram (Appendix 1, paper III). Occurrence of behaviours was observed during a 30-s period every 15 min for the entire observation period (in total 48 h) by one observer.

3.2.5 Nest quality (study 3)

In study 3, nests were scored every 3rd day from day after mating until parturition, and on days 0, 1, 2 and 3 postpartum, using the naturalistic nest score developed by Hess *et al.* (2008). If bedding material was gathered to the site where the nest was situated, the bedding material was included when scoring the nests (Figure 3). Nest opacity and nest coverage were used as complementary measures of nest quality. Nest opacity indicated if the mice (female or pups) were visible through the nesting material or not, and nest coverage if they were visible above the edge of the nest or not, in both cases when seen from the side. Opacity and coverage were assessed from four perpendicular angles, resulting in two 5-level ordinal variables (*i.e.* 0=visible from all four angles; 1=visible from three angles; 2=visible from two angles; 3=visible from one angle; 4=not visible at all; Figure 3).

Figure 3. Top: Illustrations of bedding material gathered to form a nest. Bottom: a female not visible above the nest edge (illustrated with straight line) from any angle, scored as coverage 4. In the picture the edge is missing on one side, resulting in coverage 3. Arrows indicate opacity, *i.e.* how visible the mice are through the nesting material. Arrow A shows visibility from one angle and arrow B no visibility from the other three angles, resulting in opacity 3. (Photo: Elin Weber)

3.2.6 Weights and health (study 3)

In study 3, human handling according to standard laboratory routines was applied daily on days 0 to 3 to achieve a relatively high level of disturbance. During the disturbance the cage was removed from the rack and placed on a LAF-bench in an adjacent room. The female was weighed and then placed in a smaller cage while pups were individually weighed. During this separation the pups were also counted, marked with permanent ink (Promarker, Letraset, UK), checked for milk spots, and the cages inspected for dead pups which, when found, were removed from the cage. Female and litter were weighed again at weaning on day 23. To estimate the effect of disturbance a fifth group of animals, with a small amount of nesting material and no access to nest structure, was left undisturbed except for day 2 when the pups were counted and pups and female were weighed (control).

3.2.7 Pilot study (study 3)

A pilot study was conducted to examine the possibility of using thermal imaging to measure heat loss in mouse pups. During the daily disturbance from day 0-3, cages where placed under a thermal camera (ThermaCAM S60, FLIR, US) and heat radiation was measured during 1 minute.

3.3 Data analysis

3.3.1 Study 1

In total, 344 litter observations from 111 parental couples from 12 breeding groups of C57BL/6 and 146 litters bred by 61 parental couples from seven breeding groups of BALB/c were included in the analysis. All females in a breeding group originated from the same breeding pair and each parental couple contributed with between 1 and 8 litters (median 3). Litter loss referred to whole litter being lost, and was coded as a binary outcome (0=litter not lost; 1=litter lost). The risk of litter loss was modelled using a generalised linear model in the GENMOD procedure of SAS (version 9; SAS Institute Inc., Cary, NC, USA) and the clustering of litters from the same parental couple was accounted for. Fixed-effect predictors were constructed expressing strain (C57BL/6, BALB/c) and parity (primiparous, multiparous), and whether or not there was a previous 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. Model-based marginal means were calculated to estimate the effect of parity within each strain separately and transformed into predicted risks.

3.3.2 Study 2

In paper II the course of events from birth of a litter until all pups were dead were observed and described in detail. Besides describing the interactions between female and pups, other events (*i.e.* aberrant behaviours, problematic parturition) that might be relevant for pup survival were also described.

In paper III data were arranged with one observation per 15-min observation and the observation period was divided into sub-periods (48 1-h, 16 3-h, 8 6-h, and 2 24-h periods). Data were averaged for each female, sub-period and for the entire observation period, by calculating female-specific relative frequencies of all behaviours. Some behaviours occurred in low frequency and where therefore aggregated into behaviour categories (Table 2).

Table 2. *Overview of behaviours from the ethogram aggregated into behaviour categories.*

Behaviour category	Behaviours included
Parturition-related	Giving birth, Labour, Dystocia
Abnormal	Removing pup, Resting alone, Ignoring active pup, Ignoring still pup, Resting outside nest, Hunched posture, Digging, Tail chasing, Bar gnawing, Removing pup, Other abnormal
Self-oriented	Self-grooming, Resting alone, Ignoring active pup, Ignoring still pup, Resting outside nest, Hunched posture, Digging, Tail chasing, Bar gnawing
Nest building	Nest building, Move nest
Active maternal behaviour	Active with pup, Retrieve still pup, Retrieve active pup, Carrying pup, Moving pup, Active in nest
Passive maternal behaviour	Nursing, Still in nest

To examine the association between survival and predictors representing study (2A or 2B), cage design (furnished or not), and mouse strain (C57BL/6, $Hfe^{-/-}$ or $\beta 2m^{-/-}$), simple logistic regression models of litter survival (no or yes) were constructed at the female level using the Stata Logit command (StataCorp SLP, College Station, Texas, USA), containing one predictor variable at a time. To investigate the association of survival with different behaviours, ten hypotheses were formulated based on the aggregated behaviour categories as well as the behaviours nest building, being outside nest and ignoring still or active pup. A simple logistic regression model of survival was constructed for each hypothesis, containing only one of the aggregated behaviour variables. In a second analytical step, behaviour variables that were found to be significantly associated with survival at $p \leq 0.05$ were used to construct a multivariable logistic regression model of survival.

3.3.3 Study 3

Nest quality was analysed by modelling the three outcome traits (nest score, opacity, coverage) separately. Nest score was normalised by calculating the natural logarithm of the reversed original score, *i.e.* $\ln(6 - \text{score})$. It was analysed by mixed-effects linear modelling using the Stata Mixed command. There were few observations with low scores for the traits opacity and coverage, the ordinal dependent variables were therefore in both cases obtained by collapsing the two lowest levels, thus creating two variables with four levels (1, 2, 3 and 4), analysed by ordinal logistic modelling. Month, day and hour of the day were re-coded as categorical independent variables, each with four approximately equally-sized categories. Pairwise correlations among the three dependent variables were checked. Each trait was modelled to estimate the effects of nesting material and access to nest structure from 21 days before to 4 days after parturition (comparing treatments S, L, SS and LS). In all three models, day category was included, as well as potential confounders.

3.4 Ethical approval (study 2-3)

Study 2 was carried out under a project license (ref. 003758) issued by the Direcção Geral de Veterinária, the competent authority for animal protection in Portugal. Study 3 was approved by the Swedish Regional Ethics Committee for animal experiments.

4 Summary of results

This section summarises the main results of study 1-3, more details can be found in paper I-IV. An effect of strain but no effect of parity on litter mortality was found. No evidence that females actively killed their pups was found. Litter loss was mainly associated with females showing less nest-building behaviour before parturition and more time outside the nest. Females given a large amount of nesting material built dome shaped nests of higher quality. The total survival of litters in all studies is shown in Figure 4.

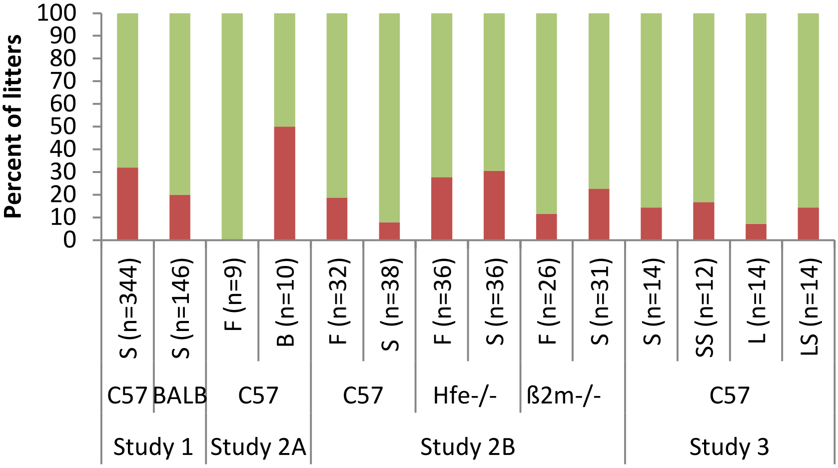

Figure 4. Overview of survival for all studies (n=number of litters born). In study 1 and 2B, litters from 1st and up to 8th and 7th parity, respectively, are shown. For study 2A and study 3, only 1st parity litters were included. Red=litters dead before weaning, green=litters survived until weaning at around 3 weeks. C57=strain C57BL/6 and BALB=strain BALB/c. B=no nesting material provided, S=small amount of nesting material provided, F=nesting material and furnishment, SS= small amount of nesting material and structure, L=nesting material, LS=nesting material and structure (details in Table 1).

4.1 Study 1 (paper I)

An overall high mortality was found in the breeding record for both strains, with a total mortality rate (calculated as loss of entire litters) of 32% for C57BL/6 and 20% for BALB/c (Table 3). A statistically significant effect of strain was found in the first parity, in that primiparous C57BL/6 females were more likely to lose their litters than primiparous BALB/c females (p=0.0028). No other effects of parity or loss of earlier litters on litter loss could be found.

Table 3. *Distribution by strain and parity, and litter loss in 490 laboratory mouse litters of C57BL/6 or BALB/c strains in study 1.*

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

4.2 Study 2 (paper II and III)

4.2.1 Paper II

In paper II three females had entire litters in which pups were never seen moving. Another three females had 1-2 pups that were never seen moving. This indicates that some pups were most likely dead at birth. While scoring interactions between mother and pups several observations of the females were made that indicated problems of giving birth. In one female, the first pup was stuck for 1 h in the birth canal during parturition. This pup was never seen moving and the female did not interact with the pup after it came loose. The female was lying in a hunched posture outside the nest for several hours before parturition (Figure 5) and was also outside the nest when the parturition started and during the following 30 min. Another female was lying outside the nest in a hunched posture for several hours after giving birth, while the pups were spread around in the nest and still alive (Figure 5). This female also moved the nest and pups to a new location in the cage about 1.5 day after parturition, but moved it back to the original site 3 h later.

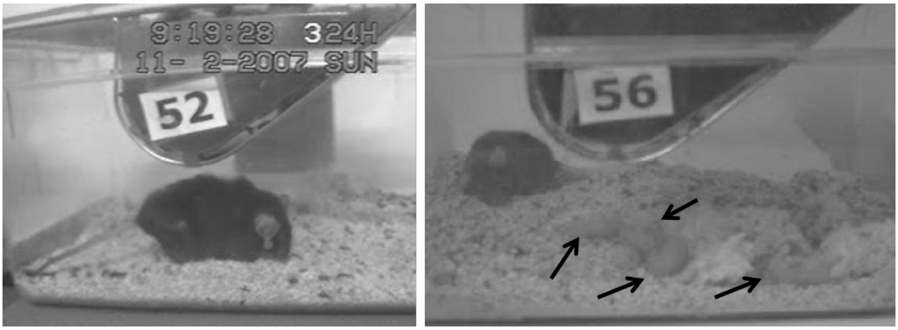

Figure 5. Images from video recording showing one female that was lying in a hunched posture for several hours before giving birth (left), and later had problems during parturition. The first pup was born 10 hours after this picture and stuck in the birth canal for 1 h after parturition started. Another female (right) was lying outside the nest for several hours while her live pups (indicated with arrows) were scattered in the nest material. (Photo: Elin Weber)

Detailed observations of interactions between mother and pups were possible to carry out for at least one pup per female in the seven females with live born pups. Females were interacting with both still and moving pups, and were observed performing maternal behaviours (*e.g.* licking and retrieving) towards dead pups (Figure 6). Females were also observed eating dead offspring (sometimes while still having live pups in the nest), but on no occasion was a female observed manipulating a moving pup that stopped moving directly after the manipulation without moving again. In most cases the pups displayed successively smaller movements until their activity was very difficult to detect and rarely seen, and the pups were finally lying still not moving anymore. Females were not observed eating pups immediately after they had stopped moving. In most cases the pups were lying still for several hours before the female started eating them (Figure 6).

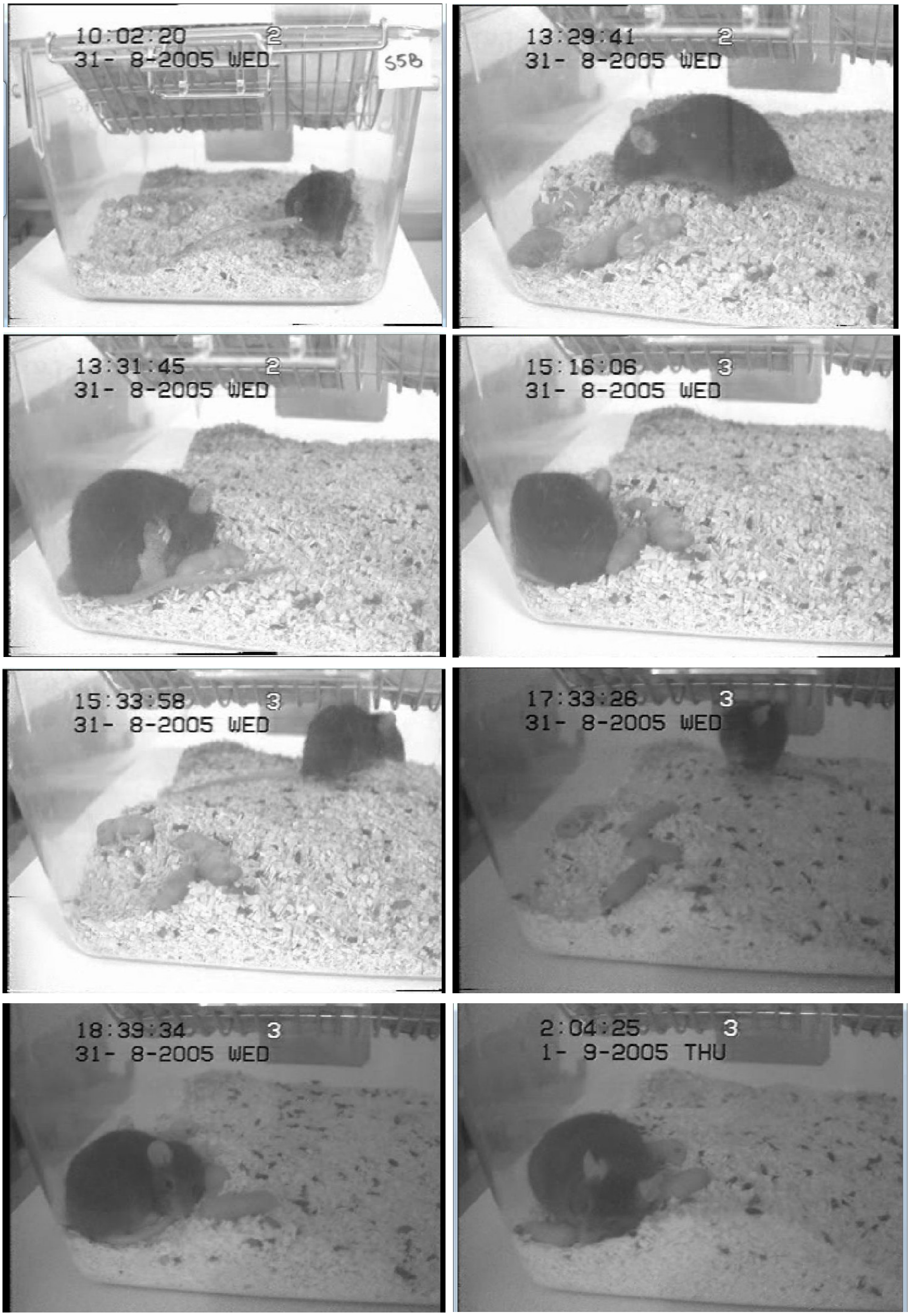

Figure 6. Images from video recordings showing a female with dead pups. Only three very small movements were detected in this litter. The pups were born 0.5 h before the first picture (top left), spread out outside the nest after birth but retrieved to the nest 2 h after birth. The pups were lying still in the nest or spread out in the edge of the nest. The female was seen manipulating dead pups, resting and sleeping in the nest with the dead pups on several occasions. (Photo: Elin Weber)

4.2.2 Paper III

Behaviour was analysed in 64 females, of these 49 successfully weaned a litter (study A n=12; study B n=37) and 15 had litters that died (study A n=5; study B n=10). Several significant associations between behaviours and survival were found (Table 4). Survival of the litter was associated with the females showing more nest-building behaviour during the last 24 h before parturition (p=0.004) and being less outside nest between 24 h before and 24 h after parturition (p=0.001). Increased litter survival was also associated with females performing more passive maternal behaviours (p=0.006) and ignoring still pups less 24 h after parturition (p=0.035). Females that lost their litters performed more parturition-related behaviours during the last 6 h before giving birth (p=0.020). A final multivariable logistic model of survival contained the behaviours “nest building” before parturition and “outside nest”, and these together accounted for 33% of the variation in survival. Predictive marginal means with 95% confidence intervals are shown in Figure 7.

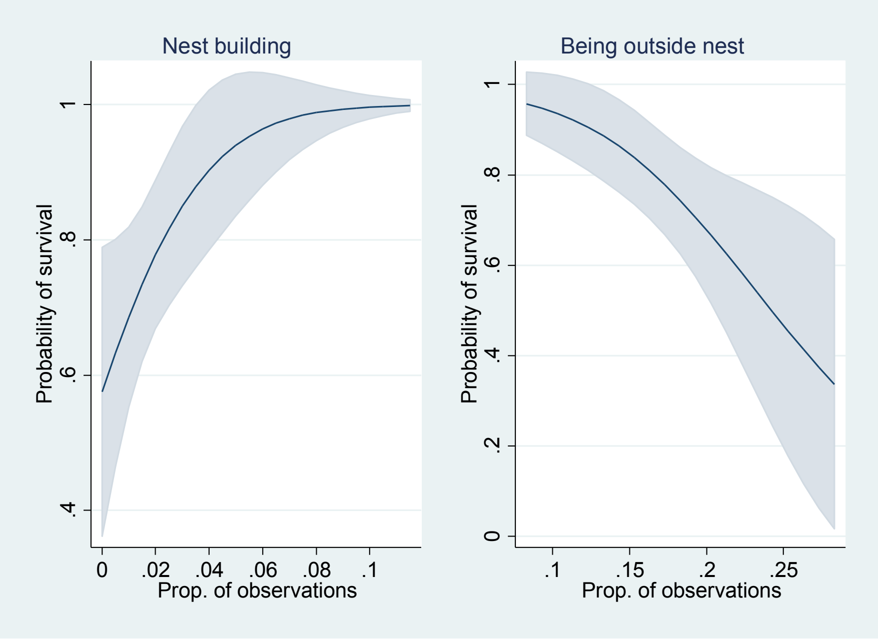

Figure 7. Predicted probability of litter survival as a function of proportion of observations with nest building and being outside nest, the two behaviours most strongly associated with low litter survival. Shaded area represents 95% confidence interval.

Table 4. *Summary of simple logistic regression models of the five behaviours for which significant linear associations were found.*

Behaviour	Coef.	Std. Err.	OR[1]	P>|z|
Nest building	76.68	26.50472	2.2	0.004
Outside nest	-26.63	7.976263	0.77	0.001
Parturition-related	-11.93	5.129796	0.89	0.020
Passive maternal behaviour	9.60	3.515016	1.1	0.006
Ignore still pup	-3.56	1.687502	0.96	0.035

[1] OR=change in odds of survival per percent unit increase in frequency of behaviour.

4.3 Study 3 (paper IV)

Of the 66 females that conceived and gave birth to a litter, 12% lost their entire litter before day 2 after birth (treatments S and SS, 15.4%; treatments L and LS, 10.7%) and another 17% lost part of their litter (1-4 pups). The majority of pups died on day 0 or 1.

Females given a large amount of nesting material (L and LS treatments) built larger nests than females with a small amount of nesting material (S and SS treatments) (Figure 8). The nests were of higher quality with regard to the naturalistic nest score, nest opacity and nest coverage.

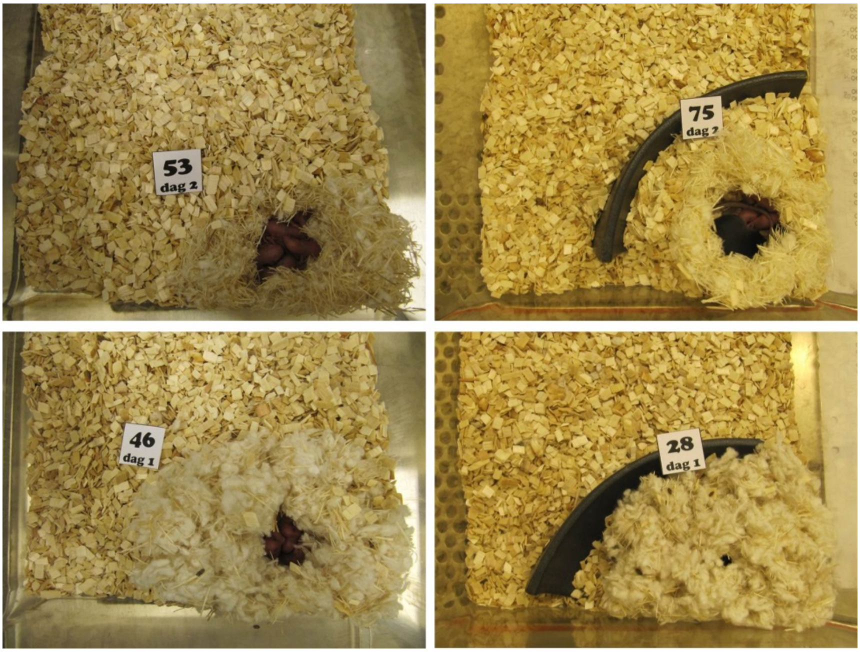

Figure 8. Nests built by females in treatments S (top left), L (top right), SS (bottom left) and LS (bottom right). (Photo: Elin Weber)

A large amount of nesting material resulted in significantly higher predicted nest scores at all day categories (p<0.001), compared to a small amount and access to nest structure increased nest score by between 0.14 and 0.20 (p=0.007) (Figure 9). The higher the nest score, the more complete is the nest, with the highest score of 5 corresponding to a completely closed dome shaped nest (Figure 8, bottom right). A large amount of nesting material decreased the odds of incomplete opacity by 98% (p<0.001) and of incomplete coverage by 99% (p<0.001) across all levels of the traits (Figure 10). Incomplete opacity indicates a nest where the female or pups are visible through the nest material and incomplete coverage indicates a nest where the female or pups are visible above the edge of the nest.

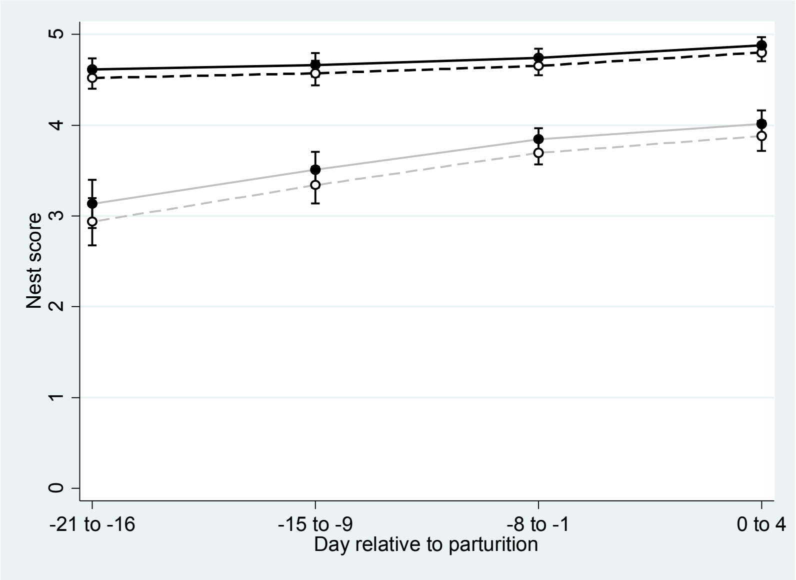

Figure 9. Predicted margins of maternal nest scores across days according to a mixed-effects regression model. Large amount of nesting material (3 nestlets) and access to nest structure (black solid line), large amount of nesting material and no nest structure (black dashed), small amount of nesting (0.5 nestlet) material and access to structure (grey solid), and small amount and no access to structure (grey dashed).

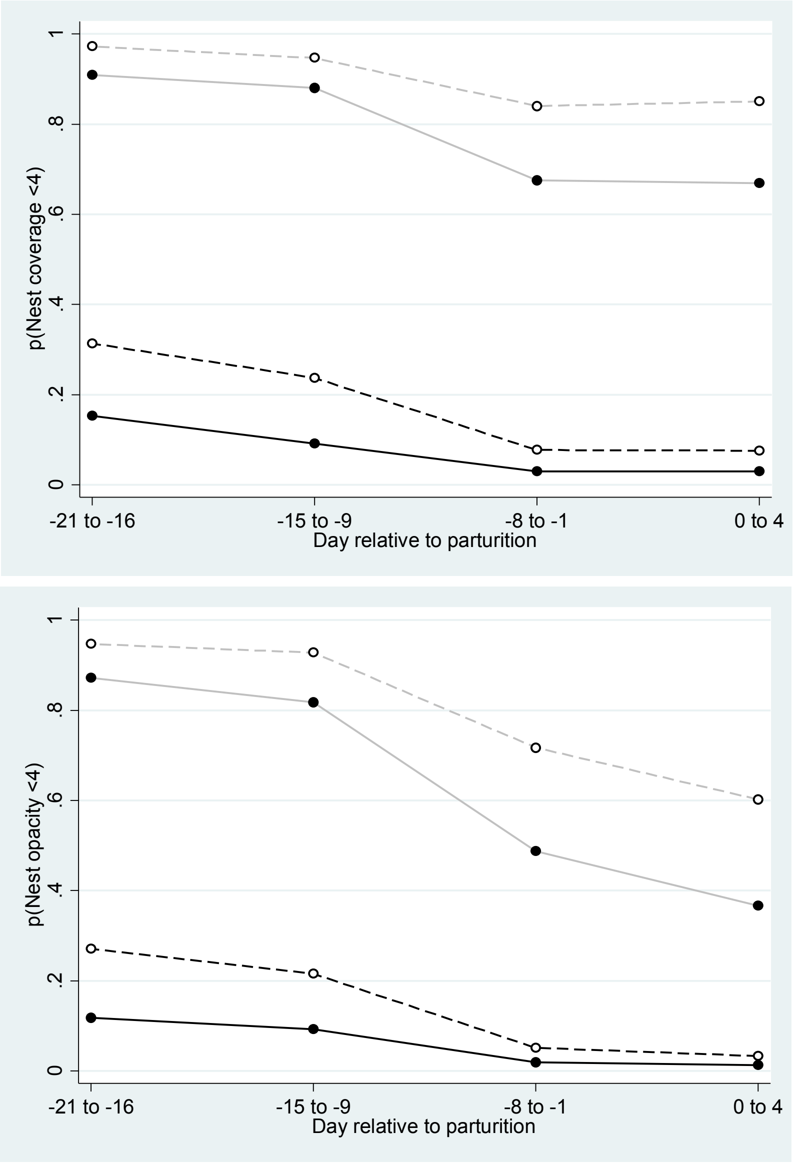

Figure 10. Predictive probability of maternal incomplete nest coverage (below score 4; top) and nest opacity (below score 4; bottom) across day categories with small amount of nesting material (0.5 nestlet) and no access to a nest structure (grey dashed), small amount and access to structure (grey solid), large amount (3 nestlets) and no access to structure (black dashed), and large amount and access to structure (black solid line), according to an ordinal logistic regression model in laboratory mice.

One female was found in a very poor condition (Figure 11) and had to be euthanized on day 1; three of her pups were found dead on day 0, one pup was alive on day 0 but found dead on day 1 and three unborn pups were found in the *post mortem* analysis of the female. Similar to study 2, dead pups were found in the outer edge of the nest (Figure 12). When the cages were inspected for dead offspring, pups were found remaining in the amniotic sac, partly eaten or intact, but no visible wounds were found in the intact pups (Figure 13). Dead pups differed from the live pups in colour (they were pale or grey), temperature (they were often cold) and activity (lying totally still). The dead pups were often found in the bedding material under the nest, but sometimes dead pups were lying with the rest of the litter in the nest (Figure 14).

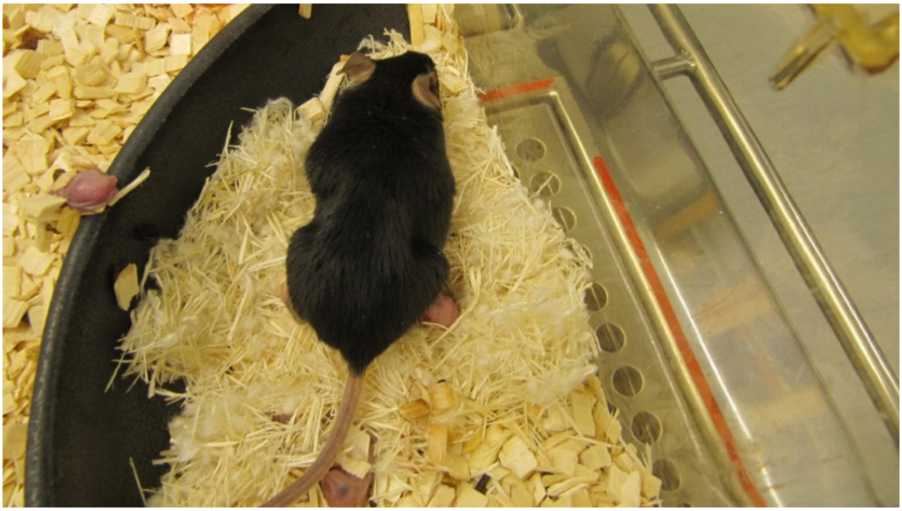

Figure 11. Female found in a very poor condition and was euthanized on day 1; dead pups were spread out around the nest. (Photo: Anne Larsen)

Figure 12. Female mouse in nest, two dead pups (indicated with arrows) have been pushed to the outer edge of the nest. (Photo: Elin Weber)

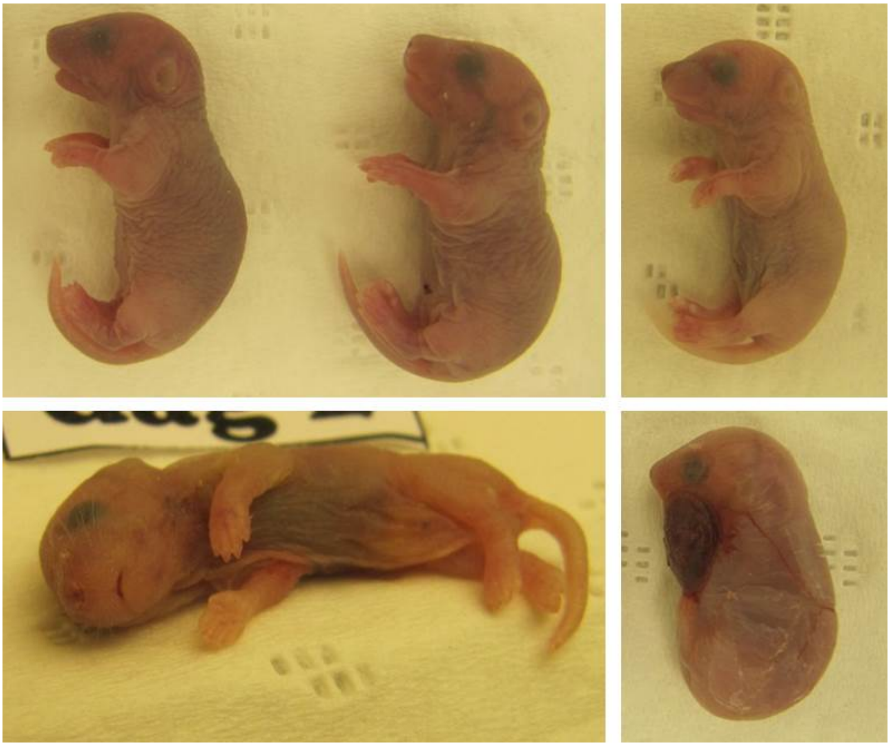

Figure 13. Dead pups from four different litters, all found without any visible wounds. Image bottom right, dead pup found inside the amniotic sac. (Photo: Elin Weber)

Figure 14. Top pictures illustrating dead pups found in the bedding material at cage inspection, bottom left picture showing dead pup found together with the live litter in the nest. Dead pups are marked with arrows in pictures with several pups or when hidden in the bedding. (Photo: Elin Weber)

4.3.1 Pilot study

Thermal imaging was feasible on newborn mouse pups as they were less mobile than adults. Measurements could not be made through the plastic cage; images were therefore obtained from above after the cage lid was removed. It was possible to follow thermal radiation both from the litter and from single pups outside the nest (Figure 15), and to detect differences in thermal radiation over the measured period of 1 min. However, if pups were not alive, they were not possible to detect since they had no heat radiation and did not differ from the surrounding bedding material.

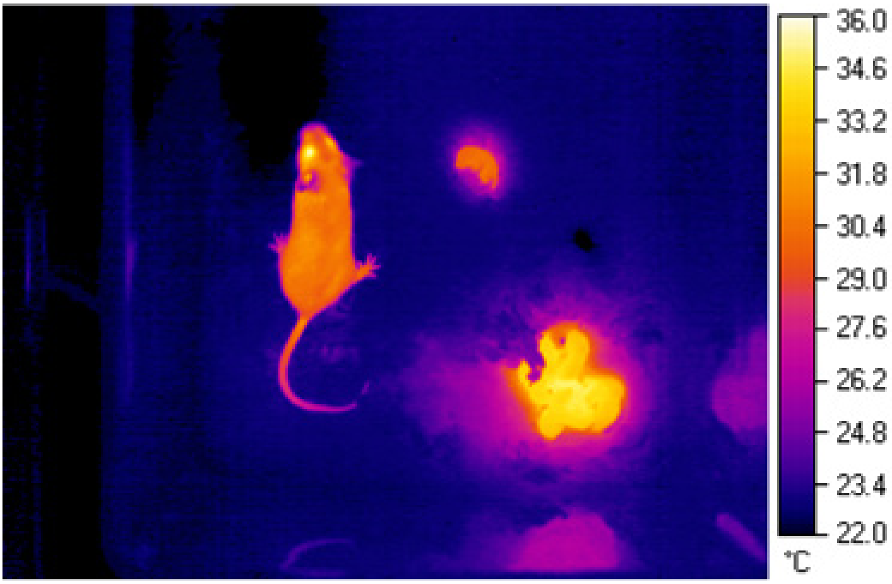

Figure 15. Caption from thermal imaging illustrating a mouse female, the litter and one pup outside nest. (Photo: Elin Weber)

5 Discussion

The focus of this thesis is to increase the understanding of pup mortality in laboratory mice, on the background of the general assumptions that it is normal for mice to lose their first litter and for some mothers to kill their offspring after birth. No support for these assumptions has been found and in the following sections the different questions raised in the included studies will be addressed, followed by a general discussion on pup mortality in laboratory mice. Problems with pups dying within the first days after birth complicates planning of research; if pups are lost there is an uncertain number of animals available for research. In several breeding facilities this has led to the practice of maintaining additional breeding animals to compensate for pup mortality, which in turn leads to an increased workload and cost. Mice are small animals and several breeding females can be kept together, and keeping additional breeding animals does not require much extra space. When discussing pup mortality with breeders and reviewing the scientific literature, it becomes clear that empirical data on pup mortality are scarce. Generally, what is described as mothers that have killed their pups often turns out to be observations of partly eaten pups or disappearance of previously observed pups without any direct observations of mothers actively killing their pups. Even in the scientific publications infanticide and cannibalism are described as causes of death without data supporting these conclusions. Evidence of poor survival of first litters is also limited.

5.1 Influence of strain and parity

When comparing the inbred mouse strains C57BL/6 and BALB/c in study 1 (paper I), a high percentage of entire litters lost (32% and 20% respectively) was found in both strains. However, high pup mortality in C57BL/6 has previously been reported (Gaskill *et al., 2013a*, 30%; Brown *et al.*, 1999, 36%; Potgieter & Wilke 1997, 22.4%). The survival of first litters differed between strains with C57BL/6 females having a higher mortality rate in their first litters compared to BALB/c. Differences between strains have been described for a wide variety of traits, including reproductive performance (Brown *et al.*, 1999; Potgieter & Wilke, 1997). However, in this study, a difference was only found in the first litter; there were no strain differences in overall survival across parities. An inability of primiparous female mammals to care appropriately for their offspring has been described, with maternal responsiveness reported to affect survival (Nowak *et al.*, 2000). Although Brown *et al.* (1999) found higher survival in second than in the first litters in both C57BL⁄ 6J and DBA⁄ 2J mice, no effect of parity in any of the strains could be found in this study. This discrepancy in results might be explained by mortality calculated as loss of entire litters in this study, compared to loss of single pups in the study by Brown *et al.* (1999).

5.2 Infanticide

Under certain circumstances it can be adaptive for a female to kill her offspring, if killing of one or more offspring increase the chance of weaning the remaining litter (Elwood, 1991). In a study investigating cannibalism in the golden hamster (*Mesocricetus auratus*), Day and Galef (1977) found that female hamsters adjusted their litter size to a specific size the first days postpartum. They concluded that it was a reproductive strategy for the females to adjust number of young to their capacity to wean them. König (1989) further found that when under food restriction, female mice killed part of their litter.

To examine if female mice killed their pups, dams were observed in detail from time of birth until the pups died (paper II). They were observed interacting with both live and dead pups, but were never observed actively killing their young. Instead, they displayed maternal behaviours with dead pups such as retrieving them to the nest, crouching over them and licking them. Some pups were never seen moving and were thus likely stillborn. Others were observed spread out inside or outside the nest and gradually decreasing movements were observed until the pups eventually stopped moving and remained still. After the pups stopped moving they were lying still for hours before the female began to consume them. To eat dead offspring could be considered adaptive; a dead pup constitutes energy and also, if dead pups are not removed from the nest site it will eventually lead to unhygienic and unhealthy conditions. In the confinement of the laboratory cage a female cannot remove the pups from the cage nor move to another environment herself. The best way to eliminate dead pups might thus be to consume them. This may also be the reason why pups are often found partly eaten. If females are housed under a normal dark:light schedule (*i.e.* not reversed) and a female gives birth during the night, dead pups might be present in the cage for several hours before laboratory personnel removes them from the cage. During the behavioural observations it was found that dead pups could be lying intact in the cage for several hours before the female started to consume them. Also in the farmed mink (*Mustela vison*) maternal infanticide has been suggested to be among the main causes of perinatal mortality. However, in a detailed study on periparturient behaviour, Malmkvist *et al.* (2007) found no evidence of infanticidal mothers.

5.3 Influence of behaviour

Several studies have investigated the effect of different factors (*e.g.* strain, housing systems, nesting material) on maternal behaviour (Shoji & Kato, 2006; Brown *et al.*, 1999) and reproductive performance (Spangenberg *et al.*, 2014; Gaskill *et al.*, 2013b; Carvalho *et al.*, 2009; Rasmussen *et al.*, 2009; Tsai *et al.*, 2003; Bond *et al.*, 2002; Eskola & Kaliste-Korhonen, 1999; Potgieter & Wilke, 1997), and differences both in terms of maternal behaviour and survival of offspring have been reported. However, paper III in this thesis is the first study in mice that compare the behaviours of females that lose their entire litters before weaning with females who successfully wean their litters. It was found that females from the two groups differed in several of the behaviours observed. Females that successfully weaned their litters performed more nest-building behaviour the day before parturition. This result is in line with previous research stressing the importance of a nest of high quality (Brown, 1953) and access to nesting material for survival of offspring (Gaskill *et al.*, 2013b). Losing a litter was further associated with females being more outside nest both before and after parturition, as well as the female being less passive inside the nest. Mouse pups are fully dependent on their mother and on insulating properties of the nest for nutrition and maintenance of body temperature; to be born in a protected environment is thus crucial for survival. To prepare a nest before giving birth and spending more time inside the nest decrease the risk of pups losing body temperature and increase survival. Being more passive inside the nest might facilitate for pups to find their way to the nipples and suckle for longer periods. Furthermore, a moderate amount of active maternal behaviour was found to be associated with maximum survival. A combination of being still inside the nest and active during certain periods may thus be optimal for proper caretaking of the pups. Licking is an example of active maternal behaviour and an important component of maternal behaviour (Shoji & Kato, 2006), both in cleaning the pups and for stimulating defecation.

During the first days after birth, external stimuli from the pups are important for maintaining maternal care (Ehret & Bernecker, 1986; Cohen-Salmon *et al.*, 1985). Females that lost their litters were found to ignore still pups more than females that successfully weaned their litters. Pups falling outside the nest have very limited abilities to move back to the nest by themselves and depend on the mother retrieving them. They emit ultrasonic sounds to induce pup approach and retrieval behaviour in the mother (Ehret & Bernecker, 1986). Outside the nest, pups rapidly lose body temperature and this might lead to the pups becoming weak and stop vocalising. The mother might thus not be triggered by sounds from the pups and it seems important that the female is attentive and notice pups that are lying still outside the nest. Thermal imaging as was used in the pilot study (study 3) might be useful to provide more insights into how rapidly mouse pups drop body temperature when they fall outside the nest.

Females that lost their litters performed more parturition-related behaviours, which might indicate problems when giving birth. In a study similar to paper III, Malmkvist *et al.* (2007) observed farmed mink and in line with our results they found birth problems to be important contributors to early kit mortality. In pigs prolonged farrowing has been reported to increase the proportion of stillborn piglets (Borges *et al.*, 2005).

5.4 Provision of nesting material

Both breeding and non-breeding mice build nests and nesting material is important for the well-being of mice (Olsson & Dahlborn, 2002). Not only the provision of nesting material but also the amount provided has previously been shown to influence nest building in mice (Hess *et al.*, 2008). In Study 3 maternal nest building was scored and supports these findings, females provided with a large amount of nesting material (3 nestlets compared to 0.5 nestlets) built larger nests of higher quality. Both the size of the nests and the nest score was improved. Providing females with a large amount of nesting material also improved the coverage and opacity of the nest walls. Mice with a small amount of nesting material were more visible both through and over the nest wall, indicating that the nest did not provide full shelter. Females provided with a small amount of nesting material weaned a slightly lower percent of their litters. Gaskill *et al.* (2013a) found a nearly 27% increase in survival when providing C57BL/6 females with nesting material. However, in that study females provided with nesting material were compared with females housed with no nesting material, in contrast to this study where all females had access to nesting material, but the amount provided differed between treatments. Gaskill *et al.* (2013a) also monitored breeding mice over a period of 6 months, resulting in several litters per female compared to only one litter per female observed in study 3.

5.5 General discussion

5.5.1 Pup mortality

In discussions on pup mortality it is obvious that many people breeding mice have the impression or are even convinced that female mice kill their pups, but when asking if they have witnessed the actual killing, few can confirm and describe what they have seen. It is not surprising that the assumption of killing is made since pups generally are found partly eaten or females are found eating them. During behavioural observations (paper II), females were sometimes observed eating a dead pup while simultaneously nursing the remaining live litter. It is difficult to map the occurrence of mouse pup mortality in laboratory research facilities. Very few papers report mortality rates and the results reported are often difficult to compare as different strains housed under a wide variety of housing environments are used (*e.g.* Gaskill *et al.*, 2013a; Tsai *et al.*, 2003; Bond *et al.*, 2002; Brown *et al.*, 1999; Potgieter & Wilke, 1997).

A common method to measure reproductive success is to count the number of pups born and weaned over a certain time period. This gives good information on the mortality rate, but no information on when or how the pups died. In animal facilities it is important to keep track of the animals present in the different rooms and the number of animals that are available for research. Some animal facilities breeding mice use data systems to register animals. However, sometimes only pups that are found alive are registered (own observations), since dead pups do not represent a live animal present in the room or an animal that is available for research. Thus, unless there are specific reasons to follow the reproductive performance of a certain animal, if pups are not yet registered when found dead, they are simply not registered at all. This makes it difficult to systematically keep track of the number of pups found dead after birth, and gives insufficient reliable information on mortality rates. When already registered (previously alive) pups are found dead, the management system sometimes requires cause of death to be specified. In the data system used in study 3, cannibalism was listed as one of the alternatives, and in the same data system there was no alternative for unknown cause. This indicates that cannibalism is generally considered a cause of death in laboratory mice, despite the low probability that the actual killing has been observed, which gives misleading information. Using the term cannibalism when referring to the occurrence of females eating their offspring is adequate, however the observation of eating dead offspring should not be interpreted as killing if this event has not been observed.

If data are not systematically collected, there is a potential risk of assuming that it is more common for first litters to die, or that females killed their pups, and then the true causes might be overlooked. If several females are housed together, lost pups might be even more difficult to detect. Females nest and nurse communally, if one female lose a litter of three pups and another female in the cage has 10 pups, they might nurse five pups each and if the litters do not differ much in age it might not be noticed that an entire litter died. It is also commonly recommended to leave parturient females undisturbed after giving birth. This is probably appropriate to avoid extensive disturbance around parturition, as the frequency of cage changing has been shown to influence survival, with greater mortality when cages were changed once a week compared to every second or third week (Reeb-Whitaker *et al.*, 2001). On the other hand, if females are left undisturbed and cages are not carefully inspected, this will decrease the possibilities to detect females with labour problems or dead pups. Changing the cage is a far more extensive disturbance than gently handling the animals in order to verify numbers and health status. In study 3 animals were inspected daily to establish day of birth, by taking the cages out of the rack and lifting the cage lid. In most cases the females left the nest during this procedure, when not they were gently touched with a finger to make them leave the nest to enable detection of newborn pups. When pups were detected in the cage, their dam was subjected to moderate disturbance and separated from the pups for approximately 10 minutes. A control group was left undisturbed until day 2 after birth but no differences in offspring survival was found between groups, indicating that inspection of cages and handling of dam and pups the first days after birth do not influence survival of offspring in C57BL/6.

5.5.2 An evolutionary perspective

Considering the widespread belief that female mice kill their offspring and that this behaviour is “normal” for mice, it could be interesting to view the assumption from an evolutionary perspective. The processes of gestation and lactation are highly energetically expensive in mammals (Gaskill *et al.*, 2013b). Different reproductive strategies exist when producing young, some invest a large amount of energy in one offspring, and others give birth to several young. Species giving birth to several young, such as pigs and mice, also invest different amount of energy in the foetuses. Comparing the relation between body weight and offspring birth weight, a new born mouse pup constitutes a much larger energetic investment for the mother than the piglet (Algers, 1992). Considering the investments a mouse female has made in her offspring, it is therefore likely to be important for the mouse female that all the offspring survive. It seems unlikely that it is adaptive for a female to first conceive and invest energy in producing the litter, and then kill the entire litter once it is born. However, if the mother perceives the environment as very risky or there is lack of resources, the mouse female might estimate that the chance of successfully raising a litter is very low. In this case it might be a better strategy to actually kill the offspring, and instead remate and raise a litter later when the circumstances are more favourable.

There is experimental evidence of female mice (F1 generation of wild house mice, *Mus domesticus*) actively killing some of their young when given restricted access to food. König (1989) described in detail how females on three occasions were observed to pick up a pup, carrying it to an adjacent cage and starting to lick the pup vigorously and finally killing it. Only 66% of the pups survived until weaning, but none of the females killed the entire litter, usually one pup was killed per litter and day. Perrigo (1987) challenged house mice (*Mus domesticus=musculus*) to work for food and found that females completed pregnancy even at poor feeding conditions. But at the highest work levels, over 60% of the females failed to wean their litters and they killed their pups as a response to increased energetic demands. In the wild, the house mouse can reproduce under a wide variety of circumstances, and not even in the poor feeding condition presented in the study by Perrigo (1987), reproduction was inhibited. Instead, it seems to be adaptive for the wild female house mouse to adjust to current circumstances and kill part of the litter to allocate resources and secure growth of the remaining litter. However, when bred under normal circumstances in the laboratory, females generally have free access to food and the female should not need to kill pups to adjust for energetic demands.

5.5.3 Influence of environment

Several environmental factors have been found to influence reproductive performance. For example, Gaskill *et al.* (2013b) found improved pup survival when providing nesting material and Potgieter and Wilke (1997) found that different bedding materials influenced survival. Tsai *et al.* (2003) found decreased number of pups born but higher number of pups weaned in enriched cages whereas Carvalho *et al.* (2009) found no effect on survival when comparing enriched and non-enriched cages. Construction noise is generally considered to affect production in breeding facilities and when Rasmussen *et* *al.* (2009) investigated this experimentally the number of stillborn pups increased when females were exposed to construction noise. The mentioned studies all give valuable information on environmental factors that might influence survival. However, they do not give information on how the pups die. Detailed descriptive studies of maternal behaviour are important complements necessary for understanding the potential role of the mother as well as the sequence of events leading up to pup death. It should also be noted that all the above mentioned studies were carried out with mice of different genotypes and reproductive performance also varies between strains so the results described might not be applicable to other genotypes.

The highest survival rate in the studies presented in this thesis was found in study 2a where no litters were lost in the furnished environment, and the lowest survival rate was found in the same study with 50% of the litters lost in barren housing. These two treatments were the most extreme environments present. In the barren housing, a small cage without any nesting material was used, compared to a small amount of nesting material (0.5 nestlet) provided in the standard housing in study 2b. Also in study 3, only 0.5 nestlet was provided, but in this study a larger cage and another type of bedding material was provided. A furnished cage was also provided in study 2b, however the cage size was smaller than the furnished cage in study 2a and the items and nesting material provided differed. Since different results were obtained in the different studies, it is difficult to conclude which housing environment should be provided to improve pup survival. However, in no other environment the mortality was as high as when no nesting material was provided, and this result together with the results from the behaviour observations where an association between litter survival and nest-building behaviour was found, clearly stress the importance of providing nest material for parturient female mice. This results is also in concordance with Gaskill *et al.* (2013a) who found a nearly 27% increase in pup survival when providing C57BL/6 mice with enough nesting material compared to raising a litter without nesting material.

In study 2a, two different house options were provided, one PVC house and one paper house. None of the mice built nests or gave birth to their litter in the PVC house. Instead, several of the females used the PVC house for defecation and urination. A similar observation was made for a number of females provided with the ceramic structure in study 3. After cage cleaning at day 12, the females moved the nest to the opposite corner of the cage and used the area behind the structure for defecation and urination. In the wild, mice generally partition their space into different areas; they store food in one site, rest in another and defecate and urinate in specific areas (Baumans, 2010). In the small and often unstructured cage environment it is not possible for mice to divide their space into different areas. Even if they try to keep one corner as an area for defecation and urination, the soiled bedding is often spread out in the cage as the animals move around and dig, especially when the cage is inhabited by several animals. If provided with some kind of structure, it is possible for the mice to use separate areas of the cage for defecation and urination, and although they dig, they will not spread the soiled litter. When biologically relevant enrichment is provided to animals, their overall well-being can be improved (Würbel & Garner, 2007). A large cage and more furnished environment could improve well-being in laboratory mice, and might potentially also influence pup survival. In study 3, food was also provided on the floor. This might explain the lower mortality compared to litter mortality in first litters in the other studies. When food is provided on the floor it gives females easier access to energy. It also enables females to hoard the food to the nest, and that might result in less time spent away from the pups.

5.5.4 Genotype and survival

Several studies have investigated the effects of specific induced mutations on the survival of laboratory mouse pups to increase the knowledge of biological functions; these studies can give interesting insights into genes involved in reproduction. However, most of these studies are made from a perspective of understanding biological processes rather than understanding why mouse pups die. This limits the application of these results on pup mortality in breeding facilities. Despite being a problem in many breeding facilities, there are few studies of pup mortality in the most commonly used strain C57BL/6 under normal husbandry conditions. Interesting to note is that several characteristics described as problematic and leading to early pup death in mice with different gene manipulations, were also found in the detailed studies of C57BL/6 (papers II and III). This is particularly interesting since this strain is often used as background strain when genetically modifying mice. Ignoring and scattering of young described for the Gαq/11-deficient and *fosB* mice (Wettschureck *et al.*, 2004; Brown *et al.*, 1996) was also observed during the detailed behaviour observations in paper II (see Figure 5 for ignoring scattered pups in C57BL/6). Another behaviour also found to be associated with increased mortality in C57BL/6 was problematic parturitions. Females in both barren and furnished environments were observed lying outside the nest in a hunched posture for several hours before giving birth. One female was observed giving birth to a pup 24 hours after birth of the first pup. In the same female part of the litter was also seen spread outside the nest after birth. Since some of the problems are present in C57BL/6 and this strain is often used as a background strain, it could be relevant to investigate this strain more in detail.

Female mice usually give birth during the night and the parturition is seldom monitored. This results in the risk of females suffering from dystocia or other birth problems being left unattended for several hours before laboratory personal detects the problems. As can be seen in Figure 11, these females can be found in a very bad condition. Pups which are born alive might also suffer if the female is unable to nurse them, keep them warm and retrieve them to the nest. Prenatal mortality is a well-known problem in pig production where a piglet mortality of 10-15% generally is accepted as normal by farmers (Bo Algers, personal communication), as well as in the farmed mink where mortality rates of 20-25% have been reported (Schneider & Hunter, 1993; Martino & Villar, 1990). In the Swedish regulation for farm and fur animals (SJVFS 2010:15 and SJVFS 2013:16), it is clearly stated under §5 and §12 respectively that animals should be inspected daily, and that animals should be inspected more regularly when new-born and around the time for parturition. However, despite problems also in mouse breeding, there are no similar recommendations in the Directive 2010/63/EU or in the Swedish regulation for animals used in research (SJVFS 2012:26).

Many researchers are unaware of problems with reproduction in mice since they do not work with the breeding, and animals are commonly bought from commercial breeders. However, breeding of transgenic mice often takes place in-house and if problems arise they are addressed since these mice are very expensive and sometimes very difficult to obtain compared to the wild type C57BL/6. It is sometimes recommended for especially valuable transgenic strains that special considerations should be taken such as using foster mothers to ensure pup survival and to monitor parturition, in order to be able to take rapid action and foster valuable pups in case the parturient female dies or is euthanized. However, from the mouse point of view, dystocia must be painful regardless of what strain the mouse belongs to. Breeding facilities of mice contain a large amount of animals, and bred under good circumstances, the newborn mouse pups will be hidden in a nest. Dead pups are often consumed by the mother, or they can be hidden in the bedding material under the nest. Detecting dead pups and map pup mortality in mouse breeding can thus be a challenging task. However, as Morton and Hau (2011 p. 558) expressed it ”…, if an animal is not normal, it takes time to score it and to make judgements over what actions should be taken; this is the price for practicing humane science”.

5.6 Methodological considerations

Observing maternal behaviour and pup mortality in mice is challenging. Mice are small, mainly nocturnal animals that give birth to young weighing only around 1 gram and are hidden in well-constructed nests. They are also prey animals, and easily disturbed. Therefore, video observations were used to observe maternal behaviour in detail, enabling observations of undisturbed animals. However, when recording during the night, only black and white recordings can be obtained. Even though infrared lights were used, it was sometimes difficult to observe the interactions between mother and pups. When lying still outside the nest, pups were also difficult to distinguish from the bedding material and a trained eye was crucial to detect the pups. Since it was not possible to observe mother-pup interactions when females had built elaborate nests, only females housed without nesting material or with only a small amount of nesting material was observed in paper II, excluding information on how pups from dams housed with large amount of nesting material died. It would have been valuable to have recordings from inside the nests. That might be possible if females are only provided with a shelter, however as soon as mice are given nesting material they build elaborate nests, and it will thus be difficult to place a camera inside a nest without the female placing nesting material in front of it.

In study 2A and 2B four cages were recorded simultaneously, *i.e.* one female was in view for 30 sec followed by being out of view for 1.5 min. This was a limitation during the detailed behavioural observations in paper II. However, the females were observed for several days, and despite being out of view for 1.5 minute it seems unlikely that all incidents of active killing in all females would have occurred when the female was out of view.

It could be considered disadvantageous to use data from females from two different studies with different genotypes and housing treatments as was done in both paper II and III. However, on the other hand the results show that despite these potentially confounding effects, associations could be found between behaviour and litter survival, stressing that these behaviours are most likely of high importance for survival.

The third study differed from the other studies in that the animals were housed in individually ventilated systems and that food was provided on the floor. This was done since that was common practice in the animal facility where the study was conducted. However, this limits the possibility to compare the results with the other studies.

One intention of inspecting the female and pups from day of birth was to weigh the pups and see if there were any associations between pup weight and survival. To keep track of individual pups, they were marked with permanent ink. However, despite using ink that was supposed to be permanent and marking the pups very carefully, many females managed to remove the ink, resulting in uncertainty of identifying pups. Many pups were also already dead at first inspection of the cages, and therefore no weight measurement of these pups was achieved before they died; some pups were also eaten. For these reasons it was not possible to compare data on pup weights between pups that survived and pups that died.

In study 3, the females housed in treatments with small amount of nesting material were observed to use the bedding material when constructing their nests, resulting in relatively high nest scores when assessing the shape of the nest despite that the appearance of the nests in the two treatments (small versus large amount of nesting material) differed markedly. Therefore the categories opacity and coverage were used as complements to the naturalistic nest score developed by Hess *et al.* (2008). The results show that these scores were relevant complements to the naturalistic nest score for measuring important characteristics of the nests.

5.7 Practical implications

The most prominent practical implication of litter loss in laboratory mouse breeding is that it affects breeding efficiency. This leads to an uncertainty in number of animals available for research, thus complicating planning of experiments. When this problem is compensated for by keeping additional breeding animals it not only leads to an increased workload and cost, it also directly counteracts the 3R goal of reducing the number of animals used for research. Since it is not possible to estimate the mortality rate, breeding additional animals might also result in surplus animals that have to be euthanised.

All mice build complex nests in which they sleep and pup survival have previously been found to depend on the nest quality after birth (Brown, 1953) and provision of nesting material (Gaskill *et al.*, 2013b). The studies in this thesis confirms that mice given a larger amount of nesting material build larger nests of higher quality, and that nest building before parturition is associated with increased survival of pups. Hence, for reasons relating to animal welfare and ethics and the 3R principles, it is therefore recommended to always use a large amount of nesting material when breeding laboratory mice.

Mice in all three experimental studies were found with birth problems. Mice generally give birth during the night, which makes it difficult to monitor parturition. However, if parturitions are not monitored, this could result in females suffering for many hours before being detected. Having staff working during the night in breeding facilities, or keeping breeding animals on a reversed light:dark schedule could increase the probability of detecting females with problems during labour. Several females that had problems during labour were observed outside their nests. If females are lying outside the nest around time for parturition this could be a sign that something is wrong. Paying attention to the behaviour of females is therefore important. Cages should also be inspected carefully after parturition to enable detection of dead pups and both live and dead offspring should be recorded to keep track on the mortality rate.

6 Conclusions

- A higher survival rate was found in first litters of BALB/c mice compared to C57BL/6. However, no evidence of higher litter mortality in first parity could be found.

- When observing female mice from birth until the litter died, females were not observed actively killing their offspring.

- Survival of mouse litters the first days after birth was mainly associated with females showing more nest-building behaviour before giving birth and spending less time outside nest after birth. Survival of offspring was also associated with females being more attentive to still pups lying outside nest, and females being more still inside the nest.

- Litter loss was associated with females spending more time on parturition, and some females seem to have difficulties giving birth.

- Female mice provided with more nesting material (3 nestlets compared to 0.5 nestlets) build larger nests of higher quality. The nests were dome shaped and completely closed, and the females were less visible through and above the nest wall.

- No significant effect of housing treatment was found on survival, but a slightly higher percentage of entire litters were lost in treatments provided with a small amount of nesting material.

7 Svensk sammanfattning

Musen är det däggdjur som utgör den största andelen av de djur som används inom biomedicinsk forskning. Under 2011 användes 7 miljoner möss i försök inom EU, och sett över hela världen används omkring 25 miljoner möss. Aveln utgör en viktig del av försöksdjursanvändningen; utan fungerande avel finns det inga djur tillgängliga för försök. I många försöksdjursanläggningar där möss föds upp förekommer det att en stor andel av musungarna dör kort efter födseln. Ungdödlighet kan handla om både enskilda musungar som dör, eller hela kullar, och påverkar försöksplanering negativt eftersom det leder till en osäkerhet kring hur många djur som kommer att finnas tillgängliga för forskning; detta gäller särskilt när hela kullar dör. Ett vanligt tillvägagångssätt är att använda extra avelspar för att ta med i beräkningen att några kullar kan gå förlorade, detta löser dock inte orsakerna. För att effektivt kunna förebygga och åtgärda problem med ungdödlighet är det viktigt att känna till hur och varför musungar dör. Trots att problemen är relativt omfattande har relativt lite forskning gjorts inom detta område. Det finns ett flertal teorier om varför musungar dör, men de flesta är dåligt underbyggda och systematiska studier saknas. Det övergripande syftet med denna avhandling var därför att öka kunskapen om ungdödlighet hos möss.

En rådande uppfattning är att det är normalt för förstfödande möss att förlora sina kullar eftersom honan då är oerfaren. För att undersöka om det fanns något sådant samband användes avelsdata från en enhet med möss av två vanligt förekommande stammar (C57BL/6 och BALB/c). Att en hög andel musungar dör kort efter födseln kunde bekräftas: 32% av kullarna från C57BL/6 och 20% från BALB/c förlorade hela sina kullar. Däremot kunde inga samband hittas som styrkte att det är vanligare att förstakullen dör. Sett ur ett biologiskt perspektiv skulle det troligtvis vara ofördelaktigt för en hona att först investera i en kull, för att sedan inte att ta hand om ungarna. Modersbeteende är snarare något som förstärks ytterligare när ungarna föds, och honan tar sedan instinktivt hand om sina ungar.

En annan vanlig uppfattning är att mushonor dödar sina ungar. Mushonor äter i allmänhet upp sina ungar om de har dött, och när musungar vid inspektion av burarna hittas halvt uppätna eller saknas helt, dras slutsatsen att honan aktivt har dödat och sedan ätit upp sina ungar. Dock finns det väldigt få observationer där mushonor faktiskt har observerats aktivt döda sina ungar. För att ta reda på om mushonor aktivt dödar sina ungar gjordes detaljerade beteendestudier på mushonor som hade förlorat hela sin kull inom de första dagarna efter födseln. Honorna följdes på video från det att ungarna föddes, tills alla ungar var döda. Inga honor observerades aktivt döda sina ungar. Däremot sågs honor som interagerade med döda ungar, de både tvättade och hämtade tillbaka dem till boet. Vid flera tillfällen sågs ungar ligga utspridda i utkanten av boet, de rörde sig mindre och mindre tills rörelserna var knappt synbara och de tillslut helt upphörde att röra på sig. Honorna åt sedan upp sina ungar, men det kunde dröja flera timmar efter att de dött innan honorna började äta på dem. Några honor hade också problem under förlossningen; en hona låg utanför boet i en hukande ställning i flera timmar innan första ungen föddes och själva förlossningen var sedan utdragen med en unge som satt fast i en timme. En annan hona låg utanför boet i flera timmar efter förlossningen utan att interagera med sina nyfödda ungar som låg utspridda i bomaterialet.

En rad olika miljöfaktorer har visat sig påverka reproduktionsframgång hos laboratoriemöss, men inga tidigare studier har jämfört beteenden hos honor vars ungar dör med honor vars ungar överlever till avvänjning, vilket därför var fokus i nästa studie. Honor från två olika studier användes för dessa observationer och de var inhysta i fyra olika miljöer, från små burar utan bomaterial till större burar med bomaterial och inredning. Honor vars ungar överlevde samt honor vars ungar dog observerades från ett dygn före till ett dygn efter nedkomst och flera skillnader mellan honorna hittades. Honor vars ungar överlevde ägnade sig mer åt bobyggnad före nedkomst, tillbringade mindre tid utanför boet både före och efter nedkomst, och var mer stilla i boet efter nedkomst. Vid födseln är musungar helt hårlösa, de väger bara ett gram och är helt beroende av modern för både värme och näring. Det är därför av stor vikt för överlevnaden att musungar föds i ett välbyggt bo för att de ska kunna hålla kroppstemperaturen. Att honorna var mer stilla i boet kan också ha gett ungarna bättre förutsättningar för att dia. Honor vars ungar dog uppvisade mer beteenden som indikerade att dessa honor hade problem runt nedkomst. De ignorerade också ungar som låg stilla utanför boet i högre utsträckning. En nyfödd musunge som hamnar utanför boet har inga möjligheter att själv ta sig tillbaka till boet utan är helt beroende av honans förmåga att hämta tillbaka den. Att ungarna ligger helt stilla utanför boet kan vara en följd av att de blivit nedkylda. Det är därför viktigt för ungarnas överlevnad att honan uppmärksammar ungar, även om de inte rör sig och påkallar hennes uppmärksamhet.

Att bygga bo är ett grundläggande beteendebehov hos möss som både dräktiga och icke dräktiga möss är starkt motiverade att utföra. Även möss som har avlats i laboratorier i hundratals generationer har detta behov. Tidigare studier har visat att boets kvalité efter nedkomst är av stor vikt för ungöverlevnad, och även att överlevnaden ökar då mushonor ges tillgång till bomaterial. I den sista studien undersöktes hur mängden bomaterial och tillgång till en bostruktur påverkade överlevnad och bokvalité. I denna studie hade alla möss tillgång till en viss mängd bomaterial. Mössen i alla inhysningssystemen byggde bo, men möss med tillgång till en större mängd bomaterial byggde större bon av bättre kvalité. Endast en något högre procents kullöverlevnad hittades hos mössen som gavs den större mängden bomaterial. I studien hanterades mushonan och ungarna från dagen de föddes. En kontrollgrupp där mössen lämnades ostörda användes för att se om hantering hade någon effekt på överlevnad men något sådant samband hittades inte.

Studier utförda inom ramen för denna avhandling kunde inte styrka den vanliga uppfattningen att mushonor aktivt dödar sina ungar eller att det är vanligare att förstakullen dör. Det verkar snarare vara andra faktorer som gör att musungarna dör. Möss bör alltid ha tillgång till bomaterial. För dräktiga och digivande honor är det särskilt viktigt att de ges rikligt med bomaterial. Honor vars ungar överlevde ägnade sig mer åt bobyggnad, och honor som gavs en större mängd bomaterial byggde bon av bättre kvalitet. Problematisk förlossning var också kopplat till överlevnad. Det är därför viktigt att övervaka honor runt tiden när de ska föda, för att kunna sätta in åtgärder om honan verkar ha problem. För att upptäcka döda musungar och minska risken för felaktiga slutsatser om varför musungar dör är det också viktigt att inspektera burarna regelbundet runt tiden för förlossning. Om inte döda ungar upptäcks och avlägsnas från buren kommer honan troligtvis äta upp dem, och då minskar möjligheten att få en överblick över ungdödligheten och orsaken till att musungar dör.

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