The welfare of the laboratory mouse; Comparison in development of mouse pups in three different Individual Ventilated Cage (IVC) systems

Laboratoriemusens välfärd; En jämförelse i utvecklingen av musungar i tre olika individuellt ventilerade bur (IVC) system

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Uppsala 2013

Masterexamen i husdjursvetenskap

Studentarbete
Sveriges lantbruksuniversitet
Institutionen för husdjurens miljö och hälsa

Nr. 462

Student report
Swedish University of Agricultural Sciences
Department of Animal Environment and Health

No. 462

ISSN 1652-280X

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Nyckelord: IVC-system, Mouse pup, Body weight, Animal welfare, behaviour

Serie: Studentarbete/Sveriges lantbruksuniversitet, Institutionen för husdjurens miljö och hälsa, nr. 462, ISSN 1652-280X

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Sammanfattning

I dag är individuellt ventilerade burar (IVC-system) ett vanligt inhysningssystem för möss på försöksdjursavdelningar. IVC- systemen är utrustade med HEPA-filter och utformade för att styra både in- och utmatning av luft samt temperatur och relativ luftfuktighet i burarna. Detta gör att man får säkrare försöksresultat då det skapas en optimal miljö för mössen och minimerar risken för kontaminering av eventuella smittor inom bursystemet och från djurrummet. Syftet med denna studie var att utvärdera hälsa och välfärd med hjälp av avelshonor och ungar från två stammar av laboratoriemöss (NMRI nude och C57BL/6N) i tre IVC-system: Allentown, Arrowmight och Tecniplast. Med 18 NMRI nude (Nu) kullar i Allentown, 16 kullar i Arrowmight och 15 kullar i Tecniplast. 16 C57BL/6N (C57) kullar i Allentown, 17 kullar i Arrowmight och 16 kullar i Tecniplast gjordes mätningar på både honorna och ungarna. Ungarnas utveckling och vikt registrerades. Honornas vikt registrerades från ungarnas födsel till avvänjning dag 21. Foder och vattenkonsumtion registrerades från det honan var dräktig i andra veckan till avvänjning. Boplacering i burarna samt bobyggnad registrerades i alla system. Den största skillnaden vi hittade var Nu honornas vikt, vilken var lägre i Allentown ($p<0.01$) jämfört med Arrowmight och Tecniplast. Nu honornas foder och vattenkonsumtion i Allentown var lägre och signifikanta skillnader fanns i honornas foderkonsumtion laktationsvecka två ($p<0.05$) och vattenkonsumtion ($p<0.05$) vecka ett. Nu ungarnas vikt samt utveckling i Allentown var opåverkade jämfört med de andra två systemen. Vi hittade inga stora skillnader mellan ungarnas utveckling mellan systemen, men det finns en klar stamskillnad mellan Nu och C57. De små skillnader vi hittade kan ha påverkats av försökets upplägg, t.ex. i denna studie använde vi oss utav tillverkarnas rekommendationer med antal luftbyten i burarna.

Summary

Today the individual ventilated cage system (IVC-system) is a commonly used housing system for mice in research facilities. IVC systems have HEPA- filters and are designed to control both supply and exhausted air at cage level, as well as temperature and relative humidity in the cages. This creates an optimal environment for the mouse and at the same time a protection against the risk of cross-infection between cages and the outside environment. The aim of this study was to evaluate the health and welfare of breeding females and pups of two different strains of laboratory mice (NMRI nude mice and C57BL/6N) in three different kinds of IVC-systems; Arrowmight, Allentown and Tecniplast. The NMRI nude (Nu) females gave birth to 18 litters in Allentown, 16 litters in Arrowmight and 15 litters in Tecniplast. The C57 females gave birth to 16 litters in Allentown, 17 litters in Arrowmight and 16 litters in Tecniplast. With these litters we performed tests on both the pups and the females. We tested the pups development. The females bodyweight was registered on the same days. Food and water consumption were registered from gestation week 2 to lactation week 3. Nest location and nest score were registered in all systems. We found one difference in Nu females; they weigh less in Allentown ($p<0.01$) compared to Arrowmight and Tecniplast systems at day 14. The Nu females in Allentown ate and drank less compared to Arrowmight and Tecniplast and it was a significant difference in food consumption at lactation week 2 ($p<0.05$) and water consumption ($p<0.05$) lactation week 1. Nu pups development and weight gain in Allentown was unaffected. No big differences between pups development were found between the IVC-systems but we could see a clear strain difference between Nu and C57. In conclusion, there were no major differences between the different IVC systems in this study. The differences that we did find could have been due to this experimental setup, e.g. the different number of Air changes per hour (ACH) in the different systems. We used the manufacturers recommendations for ACH in this study.

Introduction

The house mouse (*Mus musculus*) is the most common mammal used in research today; they live their life in their home cage on research facilities. Health of the mice is important for the researcher as well as to the animal welfare; healthier animals make the research feedback more accurate and healthier mice can be assumed to have better welfare.

Housing

Housing systems for laboratory animals have been developed over the years and more modern systems such as individually ventilated caging systems (IVC) are popular today.
In the IVC system every cage has its own air circulation; hence every cage has its own micro climate. High Efficiency Particulate Air (HEPA*)* filtered air comes in to the cage from the top of the lid or from the middle back of the cage trough an air supply (figure 1). It is possible to choose how many air changes per hour the cages should have. The clean air pushes trough the cage and because it is colder compared to the warmed up air in the cage, it drops to the floor, go through the bedding, clean it from NH_3, CO_2 and reduces the humidity and then leaves the cage through the air exhaust vent. The dirty air is cleaned trough HEPA filters and goes out through the central ventilation. This is one of the benefits with IVC systems; it prevents the animals to spread air borne pathogens between the cages in the system and in the animal room (Hasegawa et al., 2003).

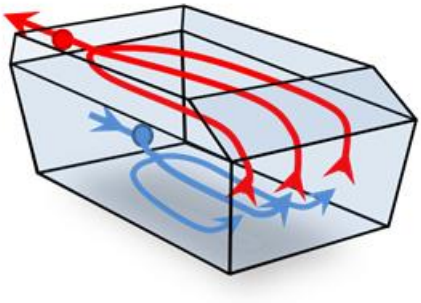

Figure 1. Example of air ventilation in an IVC cage, here from the middle back of the cage.

IVC systems have been developed to combine three main features: animal health and welfare, cost efficiency due to the limited work load for the employees and a better work environment, due to the reduced amount of allergens in the animal room. Breeding and housing mice in this environment yields low levels of NH_3, CO_2 and humidity due to the constant ventilation (Silverman et al., 2008), and leads to the possibility to clean the cages less frequently. This reduces the work load for animal care staff and may also limit stress for the animals (Reeb-Whitaker et al., 2001), especially group-housed male mice, because it is not unusual for them to fight when they get a clean environment. A study by Reeb-Whitaker et al. (2001) evaluated the micro environment (NH_3, CO_2 and air humidity) in IVC cages. They studied what the effects of reduced cage cleaning had on the health of the mice. The results showed that cage cleaning once every 14 days, with 60 air changes per hour (ACH), was an optimum for breeding pairs and breeding trios based on their health and the pup mortality.

Thermoregulation and Nesting behaviour

Due to the settings of ACH in the IVC cages, air speed at animal level can vary from approximately 0.2 m/s to 0.5 m/s (Krohn et al., 2010). This could have an effect on the temperature in the cage due to the air cooling effect from the airstream. A study by Baumans et al. (2002) evaluated mice preferences for different ACH in IVC cages. Their study showed

that the mice avoided cages with ACH of 60 and 100, and preferred unventilated cages, when they did not have nesting material. Providing nesting material made some of the mice spend time in the cages with ACH 60-100 probably because it helps them to keep thermoregulation.

Mice have their thermo neutral zone at 28-30°C but are usually kept in rooms with lower temperatures 20-23°C (William et al., 2002). Air supply is the same temperature as the room and with the air speed in the IVC cage it is possible the temperature can feel even colder (smhi.se). Nesting behaviour help the mice maintain their body temperature (Gay, 2011).

Enrichment for laboratory mice is important for their well-being; both breeding and non breeding mice build nests if they have materials to build with. Therefore providing nesting material to mice is an easy way to make the environment better for the animals living in research facilities (Sherwin, 1996). Pregnant females, prior to birth are better nest builders than non pregnant females or male mice (Gandelman et al., 1979). Nest building is an important fitness component and is positively correlated to the number of pups born and weaned. A high-nesting mouse also produces the highest-quality young, adding to the positive correlation between fitness and nest-building behaviour (Bult & Lynch, 1997).

The three Rs

The 3 Rs are developed to improve the animal welfare, to lower the number of animals used in research. A positive effect of this can be higher quality research findings.
The welfare of laboratory animals is not only important to keep the animals healthy, there is also evidence that refinement not only benefits the animals, but can also improve the quality of research findings (Smaje et al., 1998).

The 3Rs are:
1. Replacement, refers to the use of cells, tissues or organs from animals instead of the whole animal, as well as studies that do not need to use animal material at all to reach the scientific aims.
2. Reduction, refers to use methods that enable researchers to obtain the same levels of information from fewer animals or gain more information from the same number of animals.
3. Refinement, refers to improvements to scientific procedures and husbandry which minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable (World organization for animal health, oie.int/en 2011). This applies to the lifetime experience of the animal.

Refinement can be listed as:

*Using non-invasive techniques
*Using appropriate anesthetic and analgesic methods for pain relief
*Training animals to co-operate with procedures (for example taking blood samples) so the animals are less stressed
*Ensuring that the animals environment meets the animals' needs (e.g. providing opportunities for nesting for rodents)
*Environmental enrichment to improve living conditions for research animals
(The National Centre for the Replacement, Refinement and Reduction of Animals in Research. 2011)

This study was made to study the refinement parameter on the welfare of the mice in their home cage.

Mice reproduction and pup development

A mouse reaches sexual maturity when it weighs about 25 gram for males and 20-35 grams for females, which is around 45 days of age. However there are differences between different strains and in general inbred animals have later sexual maturity. Females usually reach sexual maturity before males. Female mice can accelerate their estrus cycle if they are exposed to urine from male mice (Scott and Pfaff, 1970). Mice are continuously polyestrous, which means that they come into heat at regular intervals (every 4-5 days) throughout the year until they are pregnant. When mice mate the female attracts the male trough pheromone exudation during pro estrus. Estrus and ovulation occur usually at night time. After mating a plug is created in the vagina from the male ejaculation. This plug reaches from cervix to the vulva and remains there for about 48 hours. Females are pregnant for 19-21 days and can be kept with other mice for the entire pregnancy. Female mice allow other pups to suckle her and in this way females can share the litter. Newborn pups weight around 1-1.5 g. Day 0-1 they are hairless, pink and non pigmented, a milk spot can be seen in the pups belly, eyes and ears are closed. Pigment is starting to appear at day 2 (if they're not white) and is getting darker/stronger every day. Around day 7, the first fur is shown and the pups weigh around 4 g. At day 14 the pups have their fur fully developed. Eyes and ears are starting to open and they have fully developed teeth. The pups can begin to eat solid food at two, three weeks of age. The pups reach their full grown weight at three months of age (Hall, 1994).

Aim

The purpose of this study was to compare three different IVC types, for mice, from three different manufacturers. Two strains of mice with different genetic backgrounds were used for this. The main topic in this study was to evaluate the animal welfare and the animal daily situation in their home cage with focus on animal welfare parameters. To evaluate animal wellbeing both physiological and behavioural parameters were registered in both females and pups.

Hypotheses

- There are no differences between mouse pups development between the three IVC systems

- There are no differences between food and water consumption of females, between the different cage types

- There are no differences in nest building performance and nest location between the IVC systems

Materials and Methods

Animals

We used two different strains of mice in this study, the animals were provided from Charles River, Germany.

C57 Bl/6N

C57 Bl/6N (C57) is a common mouse strain used in research. This is an inbred strain with black fur and is a common background strain for many transgenic, spontaneous or targeted mutations strains. C57 are also used to be a control strain (to other mutated strains with C57 background) in research. (http://www.criver.com/EN-US/PRODSERV/BYTYPE/RESMODOVER/RESMOD/Pages/C57BL6Mouse.aspx).

NMRI Nude Mouse (NMRI-*Foxn1*^{nu})

NMRI Nude (Nu) is a mouse with an albino background, this strain has no thymus and the homozygote animals have no fur. This defect is due to basic defects in the embryonic ectoderm. NMRI-*Foxn1*^{nu} is out bred to get a better breeding condition. Heterozygote females are mated with homozygote males to get 50% naked pups and 50% pups with fur (Gullino et al., 1976). The Nu pups are characterized by a smaller number of vibrissae with a fussy shape. This strain of mice has no thymus which leads to an insufficient immune system and are popular in research in human tumor xenografts, immunotoxicology and teratotoxicology (http://www.criver.com/SiteCollectionDocuments/NMRI%20nude24.04.07.pdf).

Room and husbandry routines

The room where the IVC systems were placed was approximately 18 m^2 (5 x 3.5 m) big. The light was on a 12 hour cycle with the light off at 12.00 to 24.00. The temperature in the room was 20.1-22.9° C and the air humidity was 28.9-62.8%. The animals were always handled in a clean area in a changing station (CS5, Tecniplast, Italy). The handler had to wear lab coat and hair net and clean plastic gloves, to get as sterile environment as possible. The changing station was always cleaned with 40 % ethanol solution after each cage and animal handling. All the animals were fed pellets (CRM, Pelleted Rat and Mouse Breeder and Grower Diet; Special Diets Services) and water *ad libitum.* All clean cages were given 140-150 gram Aspen bedding and 16 ± 0.2 gram nesting material (Sizzlenest, Datesand UK), placed at the back of the cage. Female mice were handled at cage cleaning and were weight every week during gestation. If the pups were born the same day as cage cleaning, the female got to keep her current nest for 3 days before changing the nesting material; this was made to improve the well-being and to lower the stress for the females. Food consumption was weight and recorded once a week at cage cleaning, the water was weighed and recorded and then changed (twice per week), to record the females water consumption (from the third week of gestation until weaning of the pups).

Breeding

The mice were delivered at 5 weeks of age, 36 females and 18 males of both C57BL/6N and NMRI Nu Mouse strains arrived at the animal facility. Twelve females and 6 males of each strain were placed in each of the three different IVC systems.

After one week of acclimation to the animal facility the animals were put together to mate. 12 females of each strain and system were put in pairs with a male for one week. We bred

heterozygote Nu females with homozygote Nu males to get mixed litters with both nude pups and fur pups. During the first week the females were checked for plugs at least once a day, the ones who had plugs were recorded and noted on the cage card. After one week with the male, the females were separated and put in their own individual cages. The females were weighed once every week during gestation and lactation. Mice are gregarious animal so the females who did not get pregnant were put together with other non-pregnant females to minimize stress and improve animal welfare. Those who had a litter, lived with her pups until weaning (20-22 days). After weaning, the females were placed together with their initial pair female again. Two breeding rounds were performed, so every female had two litters (if she was successfully mated both times).

Description of the IVC systems

Allentown

We used Allentown XJ cage where air was supplied via the back wall of the cage (2 cm from the cage floor) with 50 air changes per hour (ACH). The air exhaust was going out in the lid. Cage opened with two security locks, one at the front of the cage and the back of the cage. The rack contained 63 cages. The water bottles were placed on the outside of the lid on the top of the cages (figure 2).

Cage dimensions:
Length: 35 cm
Width: 16 cm
Height: 13 cm (to grid lid) 17.5 cm (to the top of the lid)
Floor area: 35 x 16= 560 cm^2
Cubic area (to the top of the lid): 35 x 16 x 17.5= 9800 cm^3
Height to feed rack (from floor without bedding material): 5.8 cm
Height to water nipple (from floor without bedding material): 6 cm

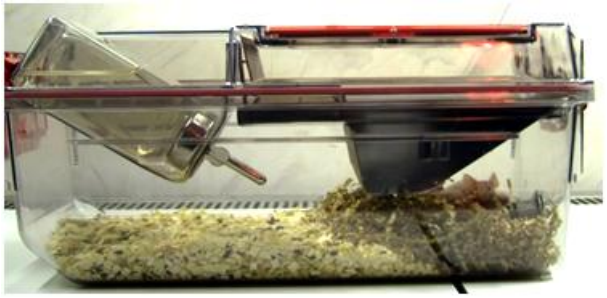

Figure. 2 Allentown IVC cage. (Photograph: Malin Wirf)

Arrowmight

The system we tested from Arrowmight was MaxiSeal IVC system. Air inlet and outlet were located in the cage lid with 40 ACH. The lid opened with a double-security lock at the front and the back of the cage top. The rack contained 56 cages. The air management unit was equipped with HEPA filtered air, the water bottle were placed outside on the top of the lid (figure 3).

Cage dimensions:
Length: 31 cm
Width: 18 cm
Height: 15 cm (to grid lid) 23 cm (to the top of the lid)
Floor area: 31 x 18= 558 cm^2
Cubic area: 12834 cm^3 (with the air space over the bar)
Height to feed rack (from floor without bedding material): app. 5 cm
Height to water nipple (from floor without bedding material): 6.5 cm

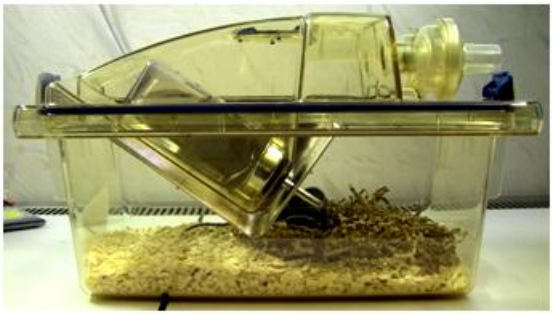

Figure 3. Arrowmight IVC cage. (Photograph: Malin Wirf)

Tecniplast

The third system that was tested was Touch slim plus rack with 70 SEALSAFEPLUS™ cages (Green-line) from Tecniplast. Air supply and exhaust were located at the top of the cage lid with 75 ACH. Clips were mounted on the front and back side of the cage top, a special rubber seal between cage and top to minimize air leakages. The water bottle was placed outside on the top of the cage (figure 4).

Cage dimensions:
Length: 32 cm
Width: 16 cm
Height: 13 cm (to lid) 16 cm (to the top of the lid)
Floor area: 32 x 16= 512 cm^2
Cubic area: 32 x 16 x 16= 8192 cm^3 (to the bar)
Height to feed rack (from floor without bedding material): 7 cm

Height to water nipple (from floor without bedding material): 6 cm

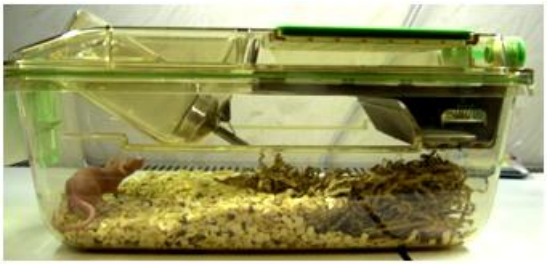

Figure 4. Tecniplast IVC cage. (Photograph: Malin Wirf)

Behaviour observations

Characterization protocol

The study was made in two litter groups so the females were divided into 2 groups, the first group were recorded in the characterization protocol (day 1-21) and the other group were weighed at day 1, 7 and 14. All the pups were weaned at day 20-22 of age and participated in the 'day 21' test (description on page 8).

All the pups in the characterization protocol were marked individually with felt pens in different colours, under the armpits day 1-7 and on their tail day 7-21 (see appendix1), body weight was measured and development was recorded at day 1, 3, 7, 14 and 21 in a protocol (protocol example appendix 2). All registrations from the mixed Nu litters were made on the nude pups. When handling the young pups (day 1-7) the female were placed in different box with aspen bedding.

Recorded every observation day

Pups location (in or out of the nest), female body weight, pup body weight and behaviour when handled observed, vocalization or not, and if the pup were active or passive during the handling.

Day 1, observations

Milk spot in the belly was recorded. A milk spot can be observed trough the skin in the belly of the young pup if they have suckled successfully.

Day 3, observations

Milk spot in the belly was recorded. The so called righting reflex was recorded; the mouse pup was placed on a flat clean paper towel on its back and had 30 seconds to turn over to the belly and paws to pass the test, a stop watch was used to record the time. If the pup did not pass the test on day three, it was re-tested day 4 and so on until it passed.

Day 7, observations

The appearance of the first fur was recorded.

Day 14, observations

Full development of the fur as well as whether or not the eyes and ears were opened, and tooth eruption and the so called clutch reflex was checked.

For eyes opened, the whole eye had to be opened but if just a gap was opened this was recorded as a gap.

The ears opens around day 14 and a cotton bud was used to separate the ear lobe from the head to see if the ear canal was open. The same cotton bud was used to separate the lips to see if the teeth where developed.

The clutch reflex was checked in all of the paws, the pup was restrained by the neck and was gently touched at the base of the paws with a cotton bud at all four legs to see if the clutch reflex was functioning. To pass this test the pups had to grip the cotton bud with their paws.

Day 21, observations

A body posture, tail position, walking were recorded when each pup moved freely in a clean empty cage. Thereafter a touch test was made with an air puff, to see if the pup reacted and a hearing test was made using the click sound of a ball pen, were also performed in the cage. The rest of the tests were done in the changing station.

The pups were weighed and then restrained to record several things; breathing condition (normal or heavy), fur condition (fur losses, wounds etc.), eye condition (clear or dirty), vibrissae (present or trimmed), nose condition (dry or snotty), teeth condition, colour on mucus membranes, tonus in abdomen and legs (legs are stiff and abdomen is distended).

Provoked biting was tested with a cotton bud (figure 5), the pup was restrained by the neck and the bud was placed at the pups mouth and it was recorded if the pup was biting. Dehydration was evaluated with the so called “tent test”. The pup is slightly pinched in the skin of the neck, and the observer scores whether or not the skin immediately returned to normal after pinching.

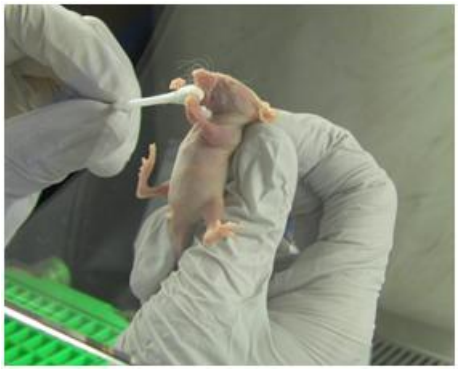

Figure 5. Provoked bite in Nu pup. (Photograph: Malin Wirf)

Grip strength was tested using a metal wire bar lid; the pup was placed on the lid which was then turned upside down approximately 40 cm above the table. To pass the test the pup had to hold on for 15 seconds. A so called position reflex test was tested with an empty plastic cage; the pup was placed in the cage and the examiners gently shook the cage from side to side and up/down and recorded if the pup parried with its legs. In the vertical pole test the pups climbing ability and balance were tested with a pole (∅ approx. 0.5 cm) covered with a soft surface (latex bandage NCH Safety). The pup was placed on the pole at a horizontal position, then the pole was tilted 90˚ and the pup had to climb up or down on the stick. It was noted if the pup used its tail as an aid while climbing. The last thing that was tested was the sense of smell, the pup was placed in a small cage with aspen bedding and a cotton bud dipped in 40% ethanol (apple cider vinegar in litter group 2) was placed in front of the pup, normal sense of smell was recorded if the pup reacted in some way; jumped, blinked with its eyes, flinched or retracted from the cotton bud.

Nest location

Every day at 8.00 (in light) and 14.00 (in dark) observations were performed in all the female cages (with pups) to record nest location. The examiner went through all the cages in the rack from the top left cage to the bottom right cage. The examiner observed the cage for one moment and noted were the nest was located and what the female was doing at that second (results from the female behaviour are not presented in this paper) (see the protocol in appendix 3). The location of the nest was recorded in one of the three zones presented in figure 6. Red spotlights were used to be able to see nest location and behaviour in the dark.

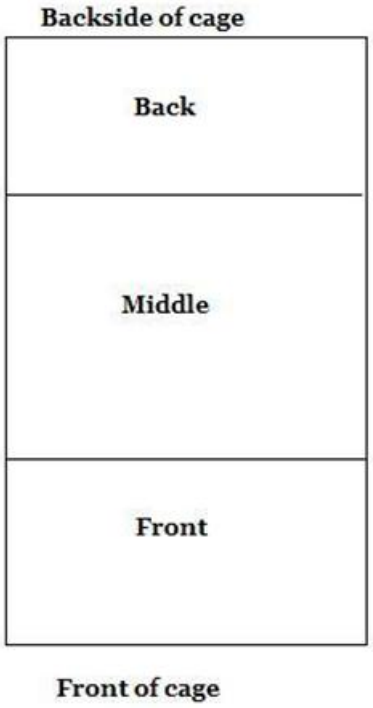

Figure 6. Schematic picture of how the examiner saw the cage when observing nest location. The nest was recorded in one of the three different zones.

Nest building performance

At day 1, 3, 7 and 14 (in second litter round) the handler had to score the females nests. Scoring scale was between 1 (flat untouched Sizzle nest) to 5 (sphere shaped nest) (see appendix 4), and based on the absence or presence of walls of the nest.

Statistics

The statistical analyses were performed with Minitab15. Differences between treatments within strains in the test were analyzed using One-Way ANOVA with Tukey´s Post-hoc test. The tested parameters were female and pup body weight, female food and water intake. Data were analyzed per cage, since the females were kept individually. Analyzes on pup body weight were performed on the mean weight per litter. Differences were regarded as significant at p-levels below 0.05. Results are presented as mean value and SD.

Results

Mouse litters

The Nu females gave birth to 18 litters in Allentown, 16 litters in Arrowmight and 15 litters in Tecniplast. The C57 females gave birth to 16 litters in Allentown, 17 litters in Arrowmight and 16 litters in Tecniplast.

Weight female mice day 1-21

In general Nu females had constantly lower body weight in Allentown cages from day one until day 21. It is a significant difference at day 14 ($p<0.01$) between Allentown (38.4 g) compared to Arrowmight (43.2 g) and Tecniplast (42.2 g) (figure 7).

There were no significant difference C57 females' weights between the systems (figure 8).

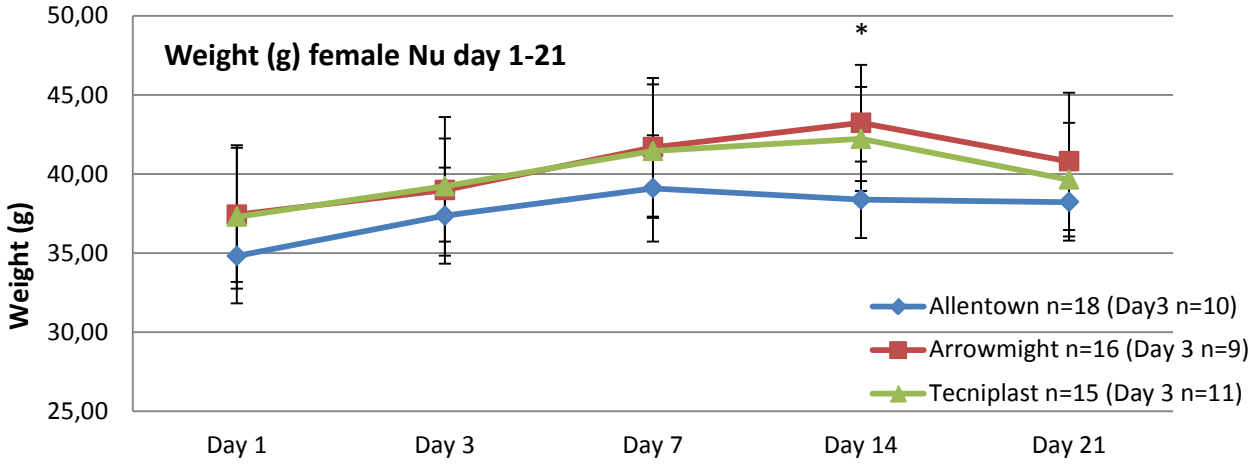

*Figure 7. The diagram shows Nu females mean body weight ± SD from day 1 in lactation until weaning at day 21. * shows significant difference between Allentown and Arrowmight and Allentown and Tecniplast.*

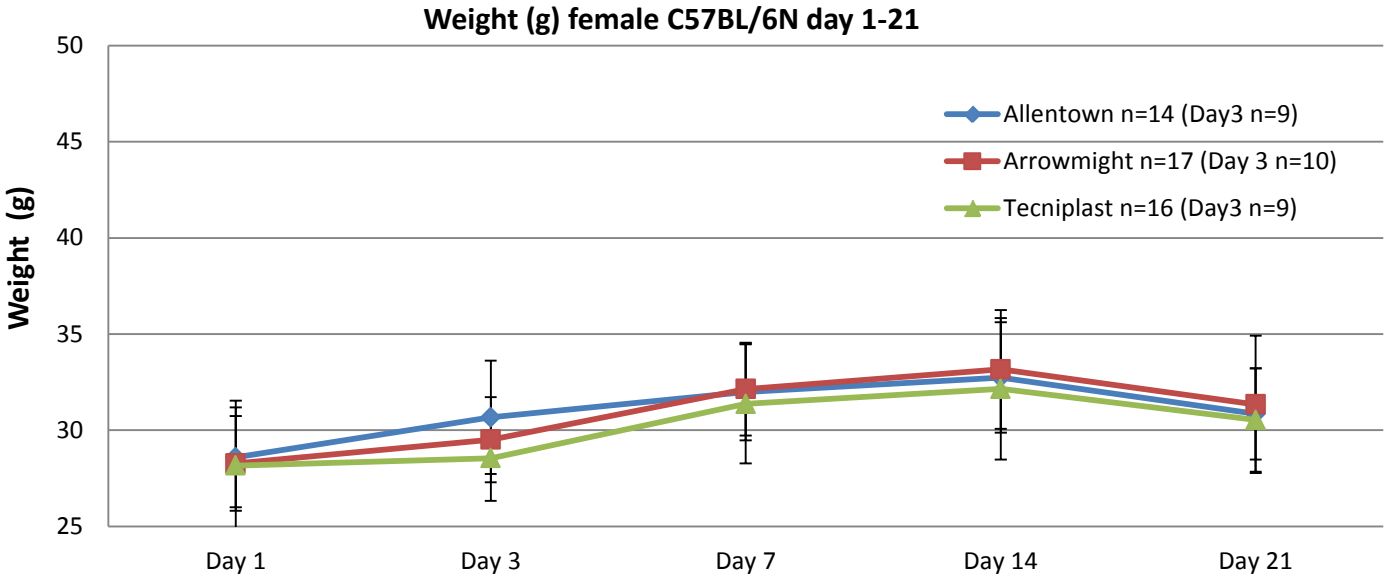

Figure 8. The diagram shows C57 females mean body weight ± SD from day 1 in lactation until weaning at day 21.

Pup weight day 1-21

It was a significant difference at day 3 ($p<0.05$) between Arrowmight (2.6g) and Tecniplast (2.2g) for Nu pups weight (figure 9).

No significant difference was found in C57 pups weight curve between the different manufactures (figure 10).

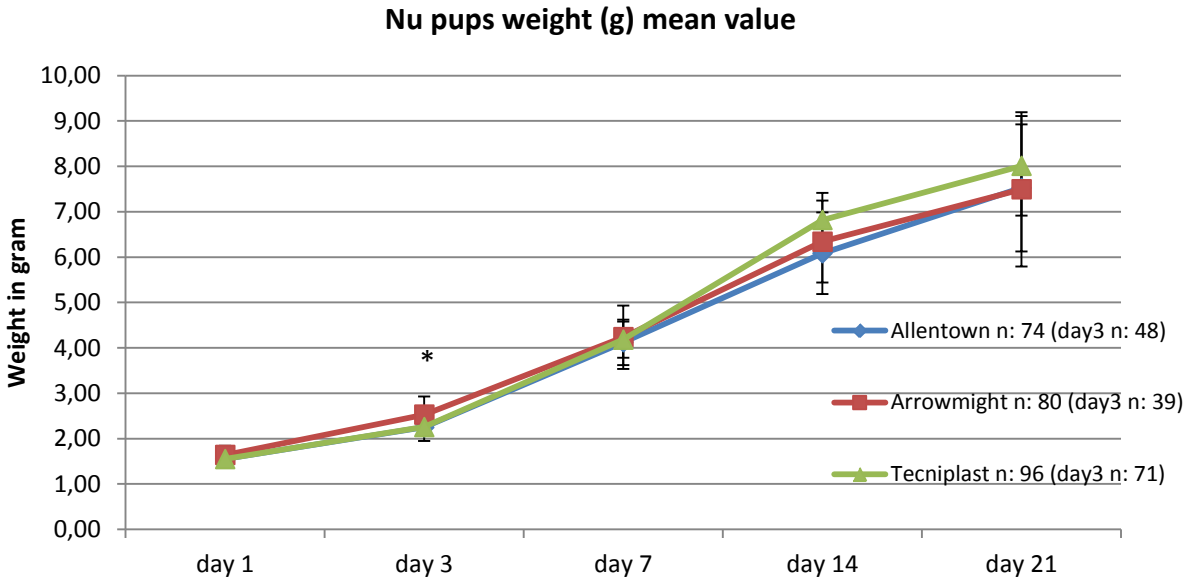

Figure 9. The diagram shows the naked Nu pups mean body weight ± SD from day 1 in lactation until weaning at day 21. Day 3 has fewer registrations because of our protocol. shows significant difference between Arrowmight and Tecniplast.*

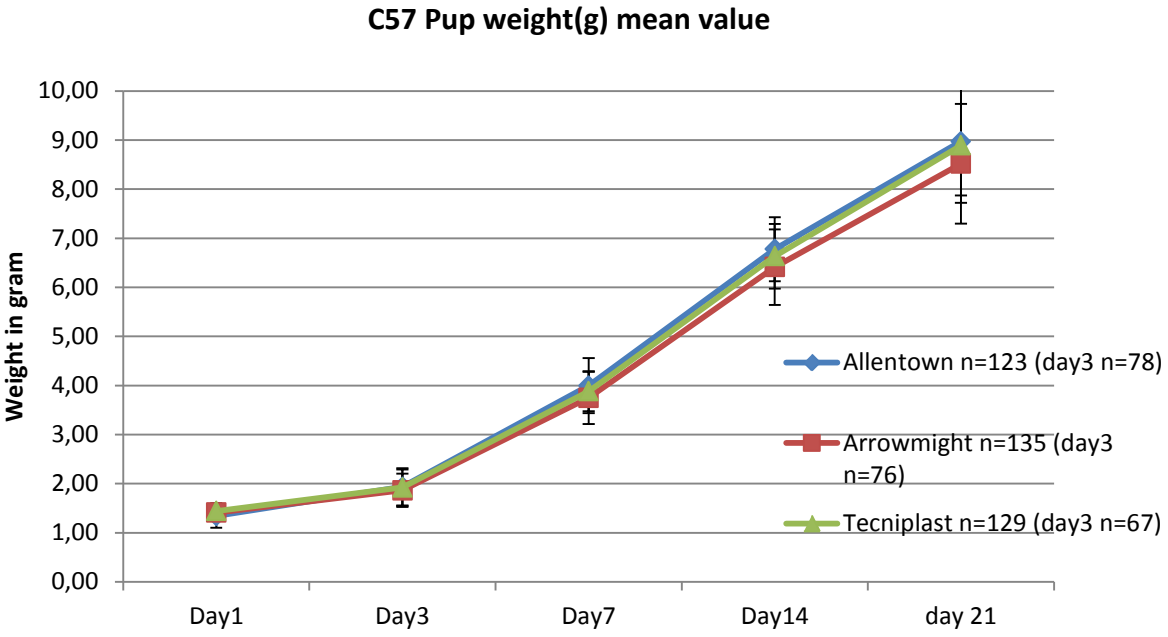

Figure 10. The diagram shows C57 pups mean body weight ± SD from day 1 in lactation until weaning at day 21. Day 3 has fewer registrations because of our protocol.

Food consumption

Female Nu mice in Allentown had lower food consumption from gestation week 2 to lactation week 3. There was a significant difference between Allentown (15 g) and Tecniplast (16.8 g) in Nu female food consumption week 2 ($p<0.05$) (figure 11).

No significant differences were found in C57 female food consumption between the different IVC manufactures (figure 12).

Food consumption is probably affected by the pups in week 3, and shows a clear strain difference in food consumption. Nu females with pups eat 19 g (mean value) whilst C57 females with pups eat around 15 g (mean value).

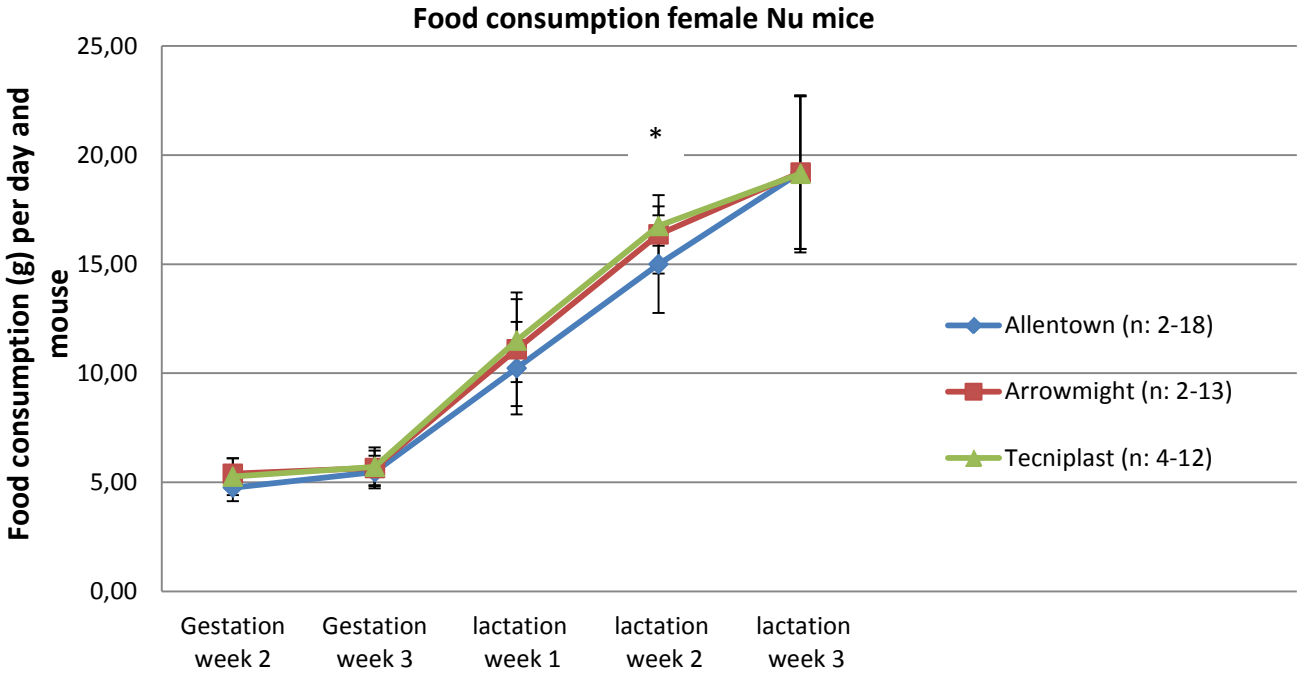

*Figure 11. Food consumption in Nu female mice and the different cage manufacturers. The diagram shows female mean food intake ± SD from gestation week 2 until lactation week 3. * Week with significant difference between Allentown and Tecniplast.*

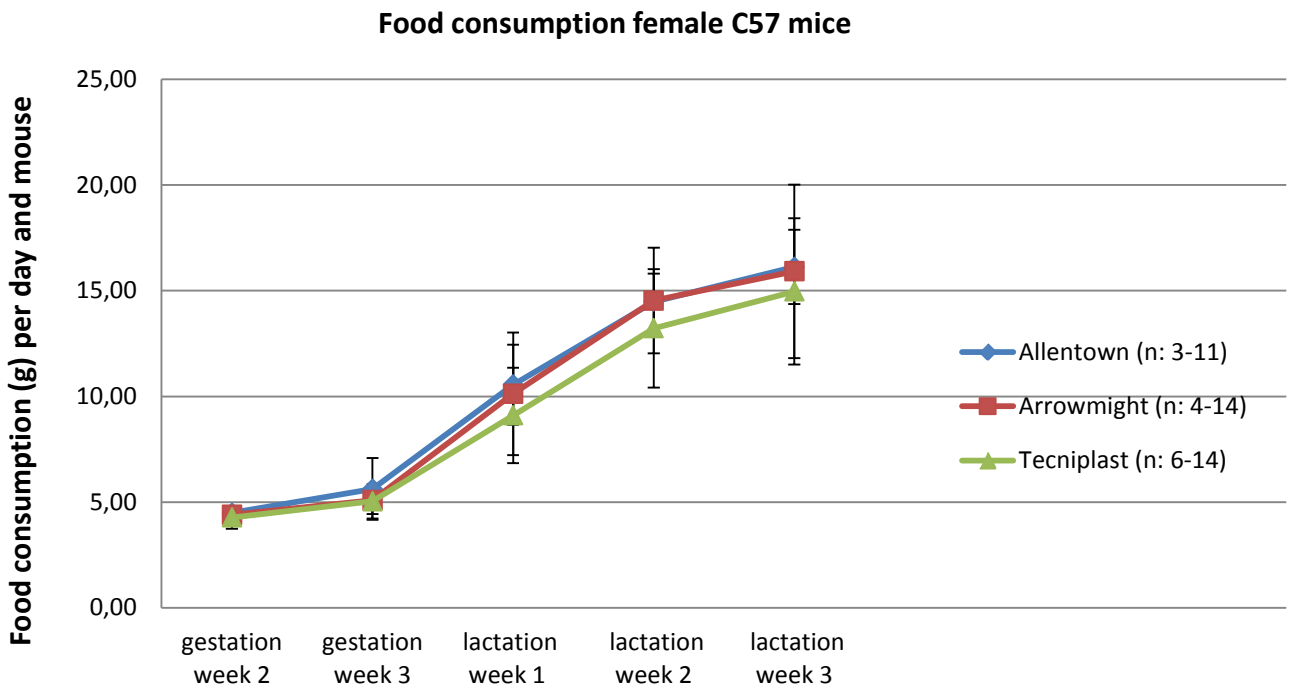

Figure 12. Food consumption of C57 female mice and the different cage manufacturers. The diagram shows female mean food intake ± SD from gestation week 2 until lactation week 3.

Water consumption

There was a significant difference between Allentown (12.8 g) and Tecniplast (14.3 g) in Nu females water consumption lactation week 1 ($p<0.05$) (figure 13).

One difference was found in C57 females water consumption, at week 3 the females in Tecniplast drank more (26.2 g) compared to Allentown (22.5) and Arrowmight (24 g) (figure 14).

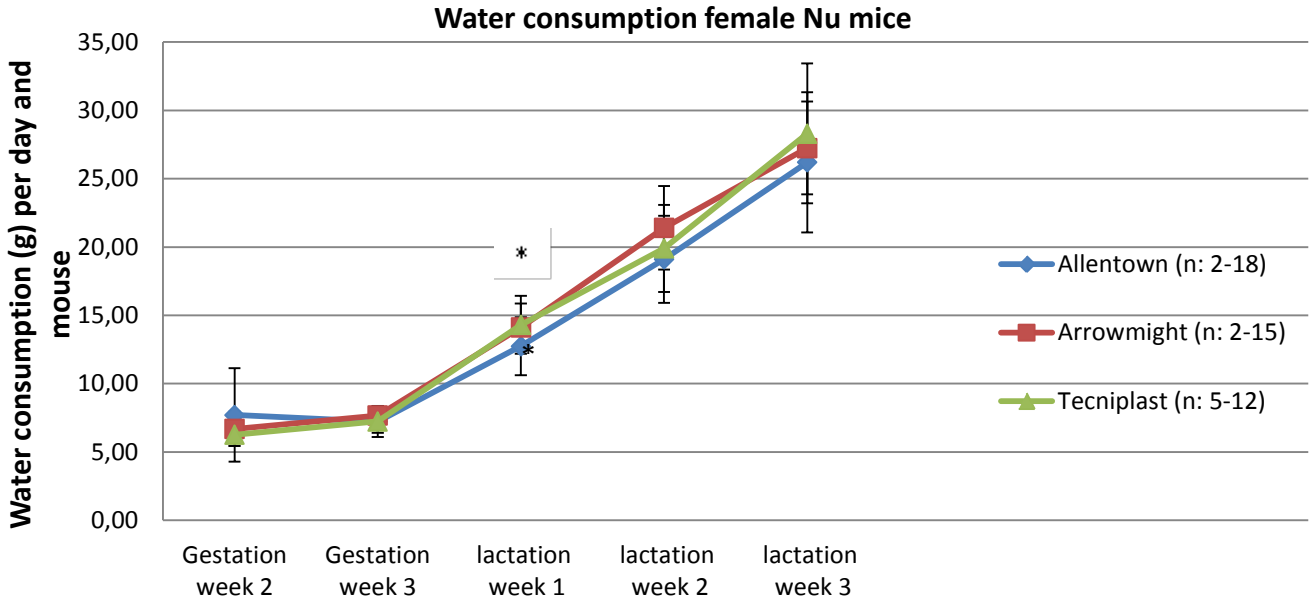

*Figure 13. The diagram shows Nu females mean water consumption ± SD from gestation week 2 until lactation week 3. * Week with significant difference between Allentown and Tecniplast.*

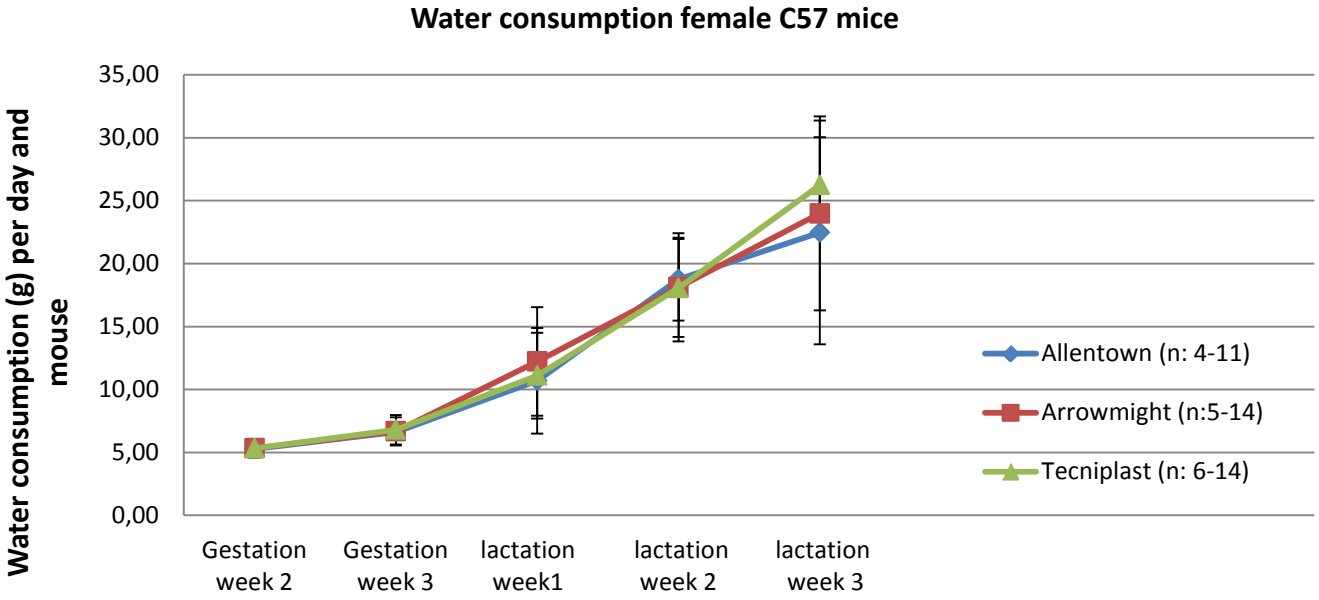

Figure 14. The diagram shows C57 females mean water consumption ± SD from gestation week 2 until lactation week 3.

Nest building performance

There were few observations at several time points which make it difficult to draw any clear conclusions from these results. No statistical analysis has been done of these results because of this.

But in general, nest score declined as the pups grew older, in both strains (figures 15 and 16). Nu female build the best nests at lactations day 1-3 with a mean value of approximately 4.6-4.9 in Arrowmight compared to 3.8-4.2 in Tecniplast.

C57 female build the best nests at lactation day 3 in Arrowmight and Tecniplast with score 4.5 and 4.6. In Allentown cages females had the best score at day 1 with a mean value of 4.3. Allentown has the highest score at day 14 with 3.4.

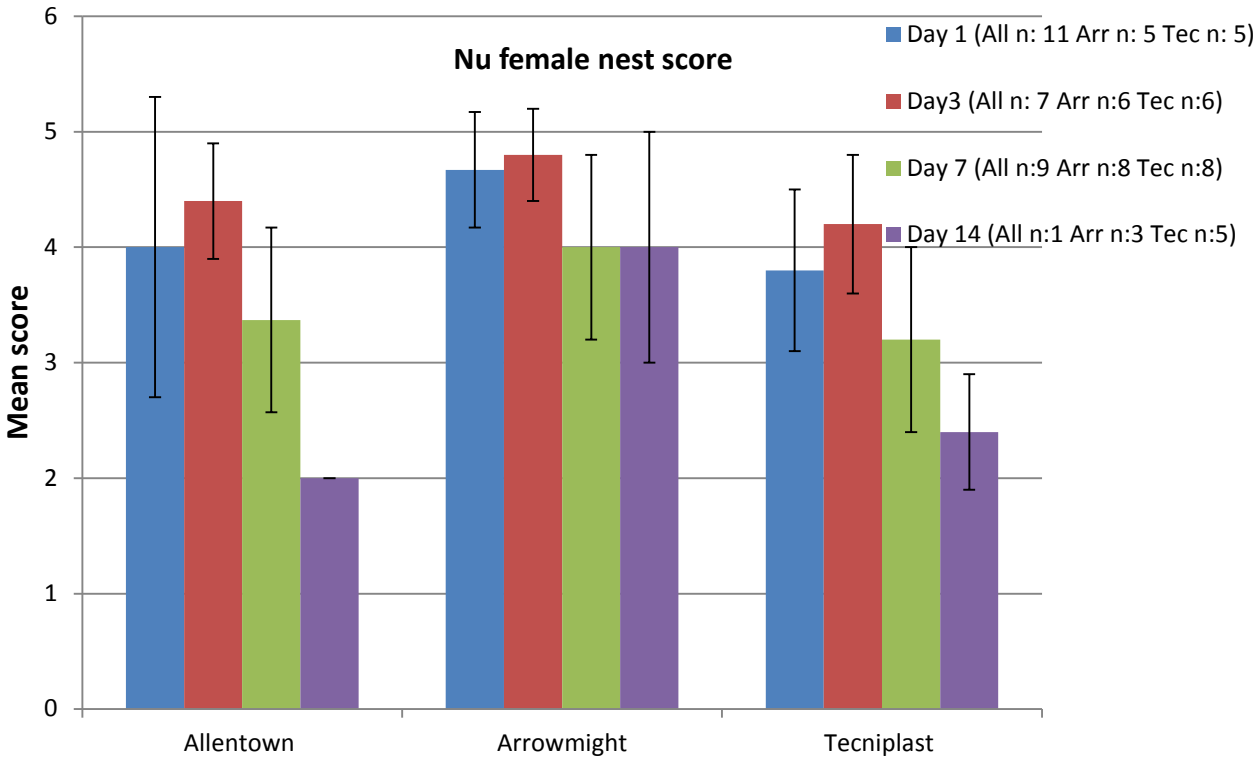

Figure 15. The graphs show scoring of the nests built by the Nu female mice in the three cage systems, the scores are a mean value by all the mice at the specific lactations days 1, 3, 7 and 14 in the different ICV systems. The SDs and total number of observations in the single recording point are also shown in the graph.

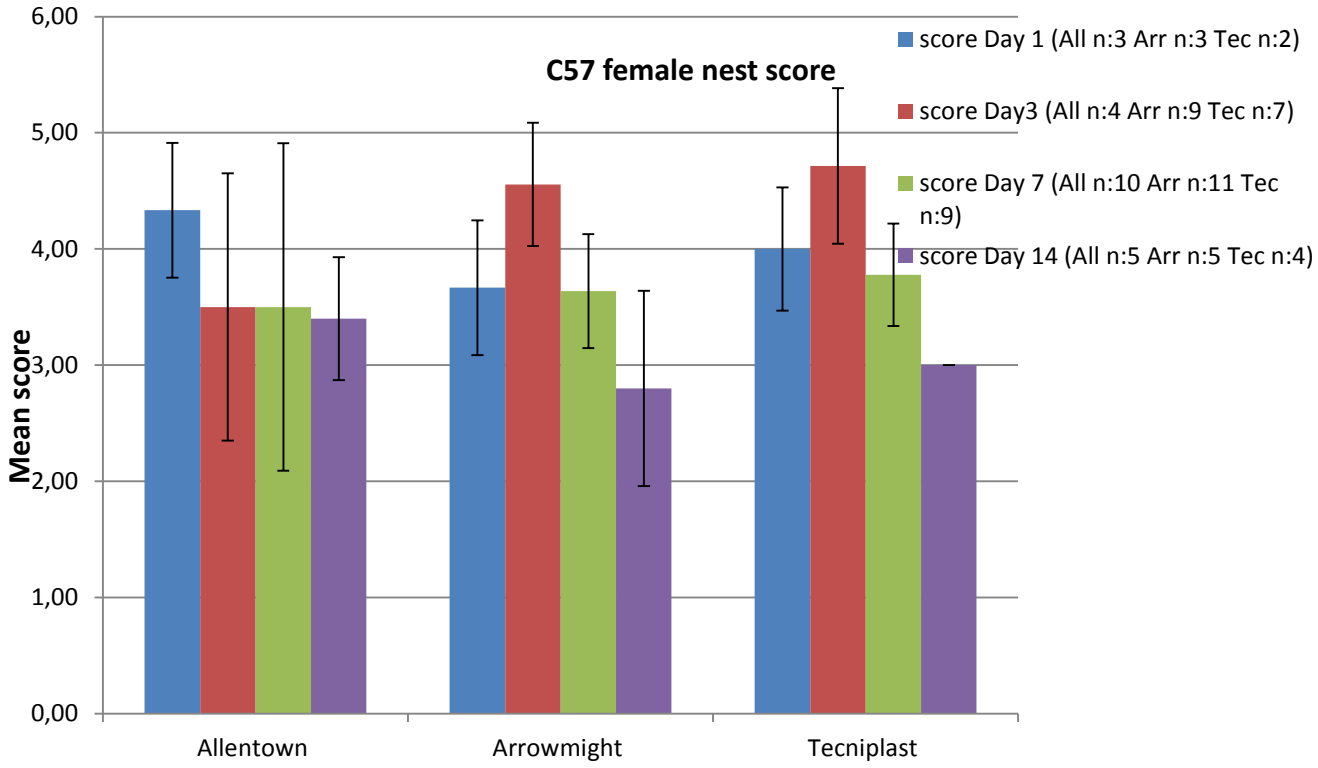

Figure 16. The graphs show scoring of the nests built by the C57 female mice in the three cage systems, the scores are a mean value by all the mice at the specific lactations days 1, 3, 7 and 14 in the different ICV systems. The standard deviations and total number of observations in the single recording point are also shown in the graph.

Nest location in cages

´Back' was the most popular location to build the nests in all the systems and both strains (figures 17 and 18). A few females placed the nests in the middle or in the front of the cage in all three IVC systems as well.

C57 females tended to place their nests in the middle of the cage during the whole lactation period more often in Tecniplast (3-47%) compared with Allentown (0-25%) and Arrowmight (3-29%). In all systems some female placed their nests at the front of the cage.

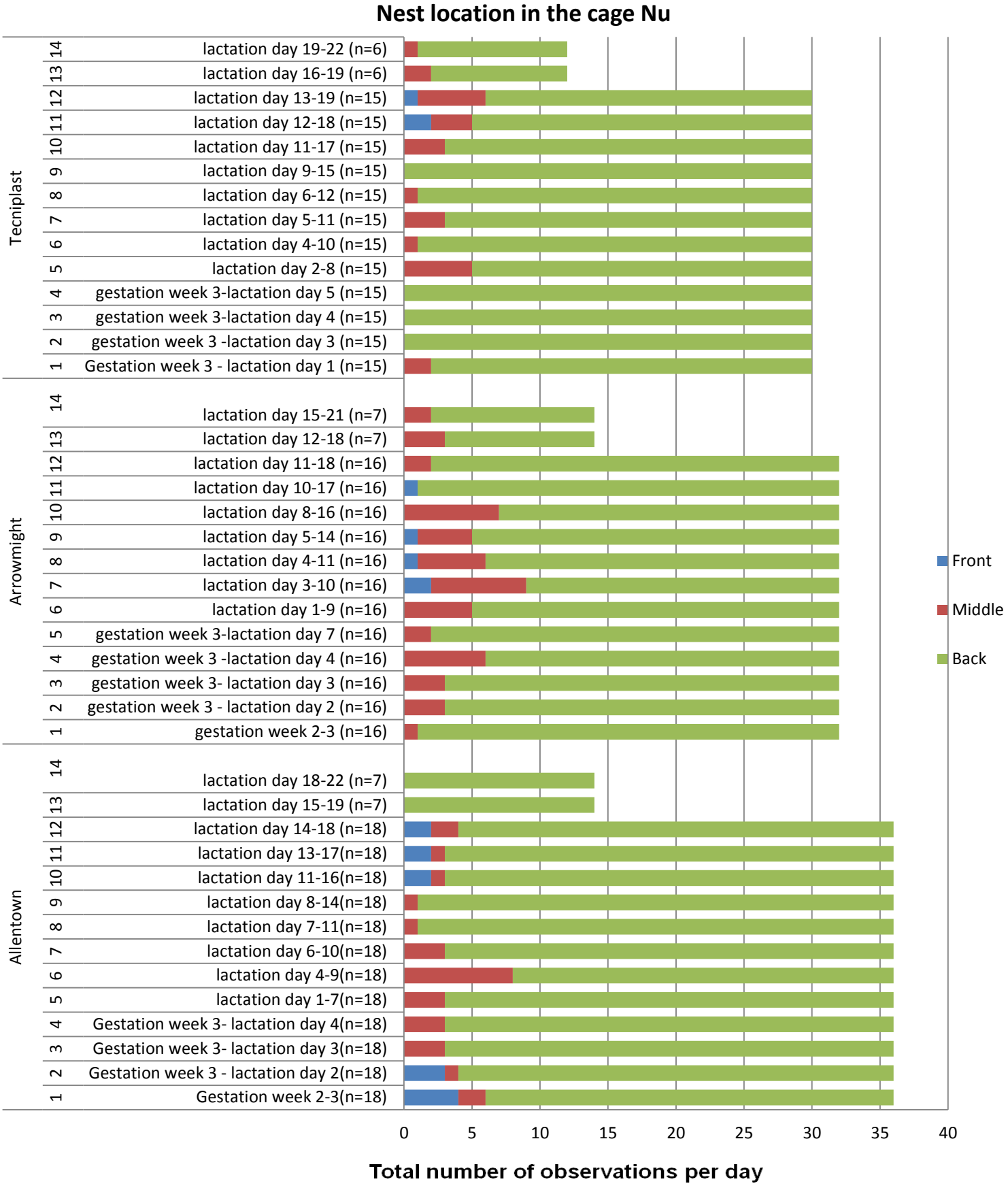

Figure 17. The diagrams show the total number of recordings of nest location in the cages (front, middle and back) in the three IVC systems. Recordings were done from gestation week 3 until lactation day 22 two times per day (total 14 recording days). The Nu mice had the most litters born in Allentown, which is why there are more recordings in this system.

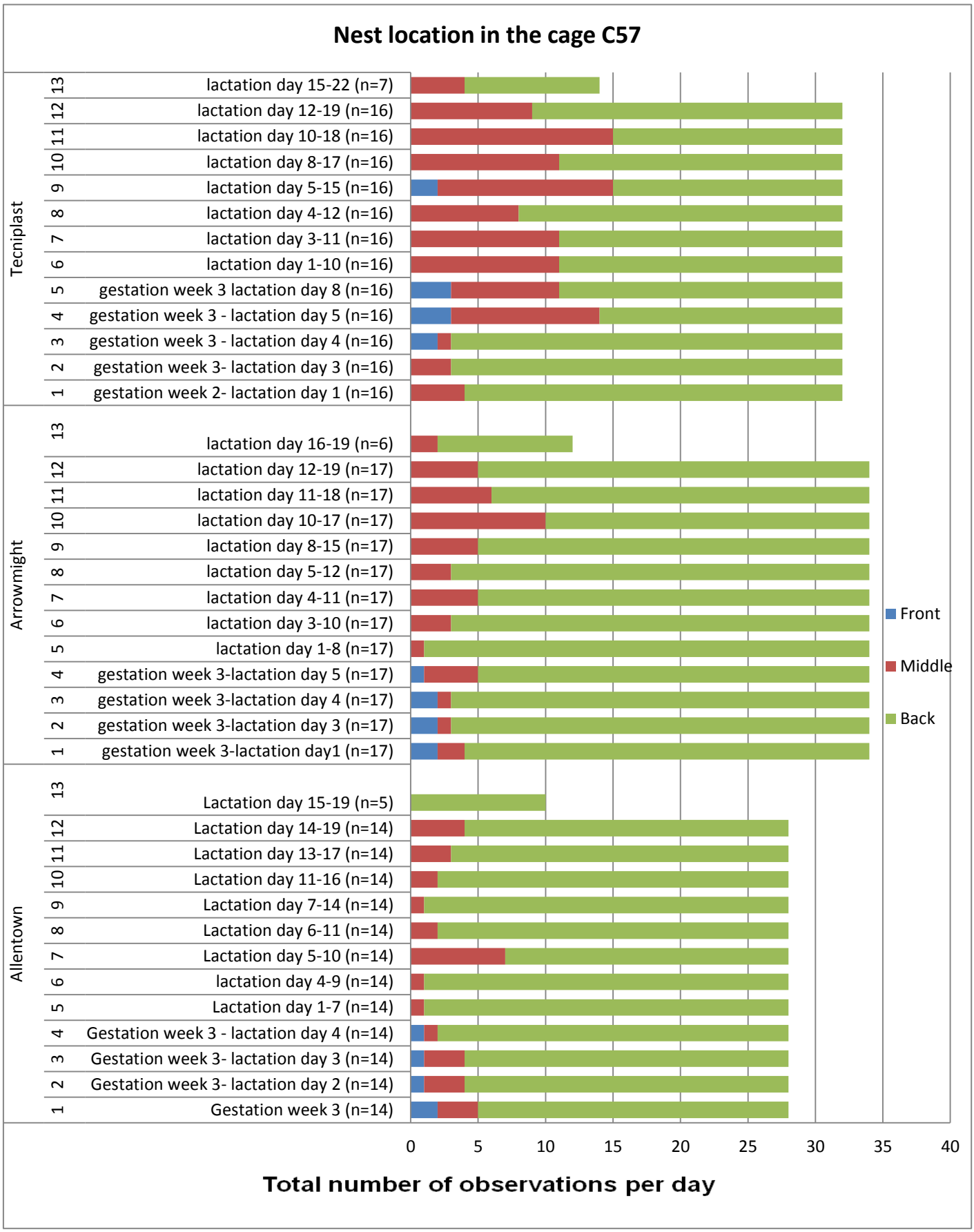

Figure 18. The diagrams show the total number of recordings of nest location in the cages (front, middle and back) in the three IVC systems. Recordings were done from gestation week 3 until lactation day 22 two times per day (total 14 recording days). The C57 mice had the most litters born in Arrowmight, which is why there are more recordings in this system.

Day of observations in characterization protocol for pups

All Nu pups in all the system had a milk spot at day 1 and 3 (table 1). Vocalization at day 1 was lower in Allentown (57%) compared to Tecniplast (66%) but highest at day 3 in Allentown (61%). Pups in Tecniplast had lowest frequency of ear development at day 14 (23%) compared to pups in Allentown (53%) and Arrowmight (71%).

Table 1. Results of the protocol day 1, 3, 7 and 14, in per cent. Numbers of pups in the protocol from the three IVC systems are presented in brackets

Nu	Allentown (n= 47)	Arrowmight (n= 38)	Tecniplast(n= 70)
Day 1			
Milkspot	100	100	100
Vocalization	57	60	66
Day 3			
Milkspot	100	100	100
Vocalization	61	45	5
Day 7			
Vocalization	0	0	16
Day 14			
Ears opened	53	71	23
Eyes opened	43	48	36
Fur developed	Naked	Naked	Naked
Teeth erupted	100	100	100
Grip reflex (weak)	6	3	9
Vocalization	15	16	0

All C57 pups had milkspot at day 1 and 3 (table 2). Vocalization was lowest in Tecniplast at day 1 (36%) compared to Allentown (51%) and Arrowmight (40%). All pups had developed their first fur at day 7. At day 14 we found one difference between the manufacturers; ear development was lower in Tecniplast (33%) compared to Arrowmight (49%) and Allentown (58%).

Table 2. Results of the protocol day 1, 3, 7 and 14 in per cent. Numbers of pups in the protocol in the three IVC systems are presented in brackets

C57	Allentown (n= 77)	Arrowmight (n= 73)	Tecniplast (n= 67)
Day 1			
Milkspot	100	100	100
Vocalization	51	40	36
Day 3			
Milkspot	100	100	100
Vocalization	16	23	15
Day 7			
Vocalization	0	0	0
First Fur	100	100	100
Day 14			
Ears opened	58	49	33
Eyes opened	84	97	88
Fur developed	100	100	10
Teeth erupted	100	100	100
Grip reflex (weak)	8	3	8
Vocalization	27%	41%	24%

No big differences were found in day three test ‘righting reflex’ between the systems, except for the strain differences between Nu pups and C57 pups (table 3). All Nu pups had passed the test at day 5. Arrowmight C57 pups had the highest score at day 3 (61%) compared to Allentown (37%) and Tecniplast (27%), all C57 pups had passed the test at day 7.

Table 3. Results of righting reflex in accumulated percent. If the pup didn’t pass at day three, they were tested again at day 4, and so on, until every pup had passed. Number of pups is presented in brackets.

Strain Nu		**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
	Allentown (n= 47)	85	94	100		
	Arrowmight (n= 38)	90	100			
	Tecniplast (n= 70)	99	100			
Strain C57						
	Allentown (n= 77)	37	65	92	96	100
	Arrowmight (n= 73)	61	82	89	99	100
	Tecniplast (n= 67)	27	46	75	93	100

In the results from the Nu pups in the clinical examination at day 21 all systems had some pups that varied from the expected result in some of the tests. In table 4 are all the tests that deviate from the expected listed in number of Nu pups per system. In table 5 are all the C57 pups results.

Table 4. All tests performed at day 21 on the Nu pups. Under "Normal" is a list of parameters where no differences between the systems were found. The numbers in the list shows the number of pups that differed from the other ones. Number of pups in the different systems is listed in brackets. 'Vocalization' is the number of pups that did make a noise while being handled. Provoked bite is the numbers that did not bite

Nu	**Allentown (n=88)**	**Arrowmight (n=88)**	**Tecniplast (n=102)**	*Normal (everyone passed)*
Deviate tail position	0	1	4	*Body posture*
Deviate walk	0	1	1	*Sense of touch*
Hearing (no response)	2	0	1	*Body mass*
				Fur (Nu)
Dehydration	1	0	0	*Nose*
Vocalization	15	24	24	*Teeth and mucus membrane*
No provoked bite	23	30	24	*Colour on ears and tail*
Tonus in abdomen	2	4	1	
Tonus in front legs	0	1	1	
Tonus in back legs	0	0	2	
Climbing and balance	1	1	1	
Position reflex	0	1	0	
Poor vision	0	0	1	
Smell (no response)	3	6	1	
Abnormal respiration	1	0	0	
Wounds	1	0	0	
Deviate behaviour	0	2	0	
Grip strength (didn't pass)	23	30	34	
- poor grip strength in front legs	0	3	2	
-poor grip strength in back legs	23	30	34	
Eye condition (poor)	17	14	17	
Vibrissae (trimmed)	11	2	5	

Table 5. All tests performed at day 21 on the C57 pups. Under "Normal" is a list of parameters were no differences between the systems were found. The numbers in the list shows the number of pups that differed from the other ones. Number of pups in the different systems is listed in brackets. 'Vocalization' is the number of pups that <u>did</u> make a noise while was handled. Provoked bite is the numbers that <u>did not</u> bite

C57	***Allentown (n= 123)***	***Arrowmight (n= 135)***	***Tecniplast (n= 129)***	*Normal (everyone passed)*
Deviate tail position	0	1	0	*Body posture*
Hearing (no response)	0	0	1	*Walk*
Vocalization	99	109	110	*Body mass*
No provoked bite	5	7	15	*Dehydration*
Climbing and balance	0	2	1	*Colour on ears and tail*
Position reflex	0	1	1	*Fur*
Poor vision	1	0	0	*Sense of touch*
Smell (no response)	6	1	2	*Tonus in abdomen*
Abnormal respiration	1	0	0	*Tonus in front legs*
Wounds	2	0	0	*Tonus in back legs*
Stereotypic behaviour	0	1	0	
Grip strength (didn't pass)	19	44	33	
- poor grip strength in front legs	0	4	1	
-poor grip strength in back legs	7	24	20	
Eye condition (poor)	8	0	2	
Vibrissae (trimmed)	0	0	9	
Snotty nose	1	0	0	

Temperature and humidity in cages

All three IVC systems had temperature and relative humidity recordings in one cage with a probe attached to the lid, above the grid. Each of these cages contained a female with pups. All the systems had higher temperature compared to the room temperature (figure 18). Arrowmight has temperature closest to the room temperature, around 23 °C (with a peak at day 13 with 25.2 °C). Both Allentown and Tecniplast have a higher temperature from day 4 in lactation compared to the room temperature. All the systems as well as the animal room have their temperature peak from day 9 until 14. All systems as well as the animal room had their Relative humidity (RH) peak from lactation day 10 until 14. RH peak in Tecniplast day 11 (47.74) compared to Allentown and Arrowmight RH peak day 13 (56.13).

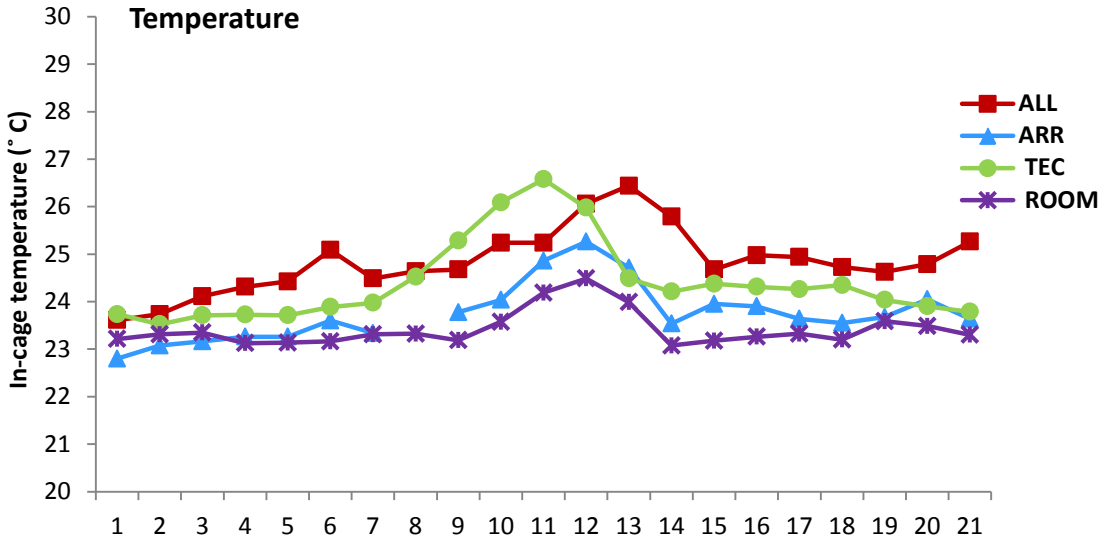

Figure 18. The figure represents 8 recordings per 24 hour, every 3rd hour, from day 1 in lactation until dav 21.

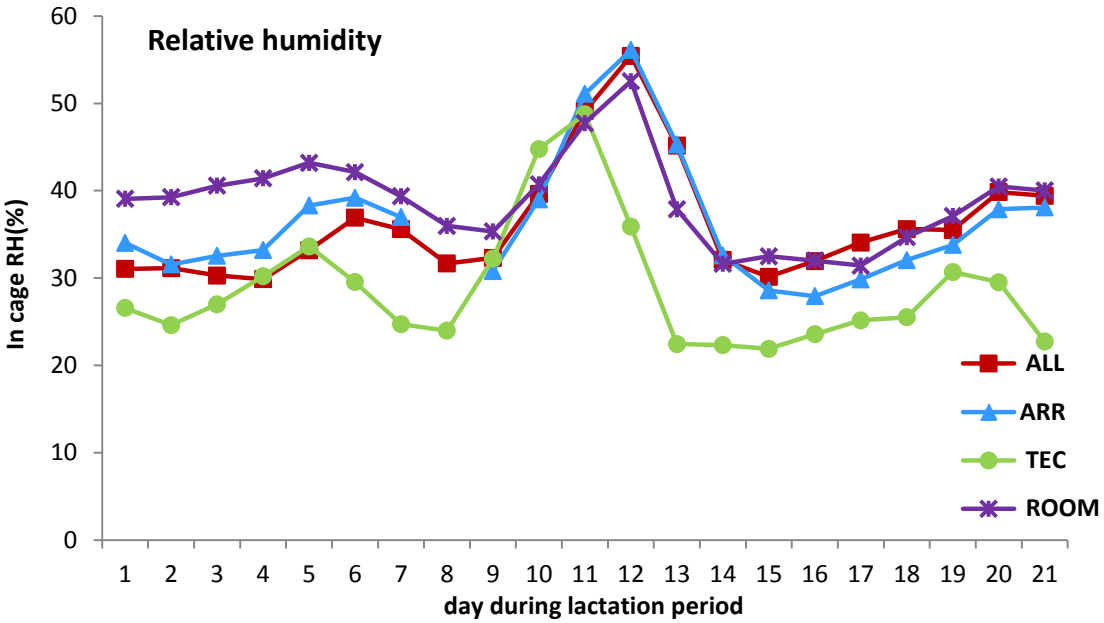

Figure 19. The figure represents 8 recordings per 24 hour, every 3rd hour, from day 1 in lactation until day 21.

Discussion

The aim of this study was to evaluate cages from three different IVC manufacturers; Allentown, Arrowmight and Tecniplast. We wanted to evaluate possible differences in the animal welfare between the systems. The study was made in two batches; this paper presents results from the first batch.

We recorded the females weight from pregnancy until weaning at day 20-22, and the pups weight from day 1 to 22. Food and water consumption were recorded in the cages with pups. We recorded the females preference for nest location as well as nest building performance in the cage, and the pups development at day 1, 3, 7, 14 and 21.

Nu female are bigger than C57 and therefore have a higher bio mass in the cage from the start and probably produces more heat. Nu pups have no fur and are smaller than their furry littermates and C57 pups; pups without fur need extra energy to keep constant body temperature so the Nu mothers surely have to produce more milk to rear the naked pups compared to the furry pups. Nu female weight was lower in Allentown from lactation day 1 until day 21 with a significant difference at day 14 between Allentown compared to Arrowmight and Tecniplast (figure 7). We did not find any differences between C57 females weight between the systems. The Nu pups weight in Allentown was not significant lower compared to the other two systems so this indicate that Allentowns Nu females was capable of produce enough milk to bring up the pups. Lactation is an energy demanding physiological process and the female have to eat (and drink) as much as half of their body weight in food in the peak lactation to manage to produce milk the pups need without losing too much body weight (Speakman, 2007). All the systems as well as the animal room hade their temperature and humidity peak from day 9 until 13 and this is likely connected to the females peak lactation. The Nu female weight in Allentown from lactation day 1 until day 21 may be connected to the significant difference between Allentown and Tecniplast Nu female food consumption week 2 and the significant difference between Allentown and Tecniplast Nu females water consumption week 1 (figure 13). This can be a reason why Allentowns Nu female weighs less compared to the other systems; they do not eat and drink enough to maintain their bodyweight but enough to bring up the pups. This is probably because the temperature in the cages is higher in Allentown in this study compared to the other two systems. With higher temperature and humidity in the cage, the females might have difficulties to get rid of excess heat that is a by-product of lactation, which can lead to decreased food intake (Król et al. 2007). Studies has showed that when female mice were shaved on their back when raising pups in 21º C cages they started to eat and drink more (Król et al. 2007). Allentown Nu female also got the lowest nest score at peak lactation (day 14) which could be caused by a too warm cage environment. With a micro-environment at 20 °c rodents use energy and have to eat more food just to maintain their body temperature. Allentowns cage temperature was around 24-25 °C with a peak temperature of 26.4 °C and around 55 RH. At high humidity levels a certain temperature can be experienced as warmer than it actually is (smhi.se). Combined with a low nest score can indicate that it is hot in the cages and the females feel like they don’t need a compact isolating nest. Mice eat less when

they are warm (Gay, 2011). Our results show that Allentowns Nu female eat enough to maintain lactation and bring up the pups but not enough to uphold bodyweight and this is probably because of the warmer temperature in Allentowns cages.

In this study we did not find any difference in nest location among the three IVC systems (front, middle and back) even though the systems have air supply from different positions. Arrowmight and Tecniplast have their ventilation from the top of the cages in the lid. Allentown has its air supply from the middle back of the cage, approximately 2 cm from the cage floor. Despite this, most females in Allentown, both Nu and C57 females choose to place their nests in the back of the cage from late gestation and throughout the lactation period. C57 females tended to place their nests in the middle of the cage during the whole lactation period more often in Tecniplast (3-47%) compared to Allentown (0-25%) and Arrowmight (3-29%) females. In all systems some female placed their nests at the front of the cage. However, this could maybe been difficult to evaluate at the observation. It could be hard to see were the nest was placed (middle or back) in the cage when the lid were on the cage. One reason why the females place their nest in the back of the cage can be that we placed the Sizzlenest at the back in the clean cage. If we had placed the Sizzle nest in the front of the cage maybe we would had some more nests in the front of the cages?

As a refinement parameter, Sizzle Nest was used in this study as nest building material. Sizzle Nest was used because it was the least dusty material used for this purpose; the naked Nu pups and naked males used for breeding do not have eye lashes and are therefore more sensitive to dust. This was also demonstrated in our tests on day 21 where more Nu pups were scored for poor eye condition, such as runny and clogging eyes in all systems compared to the C57 pups. As a result, even if Sizzle Nest is the least dusty nesting material it might still be too dusty for the sensitive Nu mice eyes.

The females build their nest with Sizzle Nest and constructed very nice nests in all the systems. C57 female had a slightly higher nest score in Arrowmight and Tecniplast compared to the females in Allentown. Female mice build nests to protect the young pups and too keep them and themselves warm (Weber and Olsson, 2008). Our temperature curve shows that the temperature was slightly higher in Allentown throughout the whole lactation period, maybe this was the reason the C57 females in Allentown build slightly poorer nests in this system.

Nu pups had slightly lower percent of eye development at day 14 in Tecniplast (36%) compared to Allentown (43%) and Arrowmight (48%). Both Nu and C57 had lower percent in ear development at day 14. Nu pups had lowest ear development in Tecniplast (23%) compared to Allentown (53%) and Arrowmight (71%). C57 pups were lowest in ear development in Tecniplast (33%) compared to Allentown (58%) and Arrowmight (49%). These are interesting results, but to know why it is like this in Tecniplast more tests need to be done.

The righting reflex was tested day 3 and everyday thereafter until all the pups in the cage passed the test. We found a clear strain difference in these results which was interesting. All Nu pups had passed the test at day 4 (except for Allentown, all pups passed day 5). C57 pups had a slower development and only 27% in Tecniplast passed day 3 compared to 37% in

Allentown and 61% in Arrowmight. It took 7 days in all systems to pass all the C57 pups. Nu pups are smaller and are hairless compared to C57 pups but with this test it seems that they have a slightly faster neurological development for some reason. This can be an important fact to think about when use of different strains for research.

In this study we handled the pups and females from day 1 until weaning at day 21 and performed cage cleaning once a week; in spite of this we had no mortality in the IVC systems litters in any of the two strains. It is often stated that mortality among litters can increase with too much handling of the young pups and cage cleaning around birth (Reeb-Whitaker et al. 2000). However that study was performed in the US and the females had no access to nesting material other than wood chips. When the female mice are used to be handled by humans and have access to nesting material as refinement parameters, and get to keeps her nest the first few days after she has giving birth, perhaps it can lower the stress for the mother and the well-being of the animals can be higher. Nest building is an important fitness component and is positively correlated to the number of pups born and weaned. A high-nesting mouse also produces the highest-quality young, adding to the positive correlation between fitness and nest-building behaviour (Bult & Lynch, 1997). At cage cleaning, it is easier to make sure that all the animals in the systems are well; the animal caretaker handles all the mice in the system and can make sure they are all right, because it can be difficult to see all the animals in the cages without taking the lid off on the IVC cage. With cage cleaning once a week the animals has a better chance to get use to the animal caretakers and to lower the stress at cage cleaning. But on the other hand, too much handling and opening the lid of the cage increases the risk for contamination since animals kept in IVC systems are protected from all the other animals in the system. This means that it is a management problem in how to combine the use IVC systems and adequate animal handling.

Conclusion

In conclusion, there were no major differences between the different IVC systems in this study. The differences that we did find could have been due to this experimental setup, e.g the different number of ACH in the different systems. We followed the three manufacturers recommendations in this study. Would we have had the same results if we had run all systems on the same number of ACH?

However we found some interesting results and it would be interesting to do more studies to evaluate these results further. In general Nu females in Allentown had a lower body weight throughout this study but the pups and the C57 females and pups in Allentown were unaffected. We have some strain differences between Nu and C57 females as well as Nu and C57 pups development, there are no big differences in development between the systems.

We found one difference in Tecniplast; the system had the lowest results at day 3 righting reflex test as well as lower ear development at day 14 in C57 pups as well as Nu pups. It would be interesting to do further studies to evaluate for example sound level and light differences between the systems.

Acknowledgements

Thanks to Monica at the animal facility of S:t Eriks Ögonsjukhus for helping us with this study.

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Appendix 1.

Mouse pup marked with colour felt pen in their arm pits, seen upside down. On their tail with the two top colours.

1	2	3	4
Front OO Back OO	Front OO Back OO	Front OO Back OO	Front OO Back OO

5	6	7	8
Front OO Back OO	Front OO Back OO	Front OO Back OO	Front OO Back OO

9	10
Front OO Back OO	Front OO Back OO

Appendix 2.

Example of the Characterization protocol.

Observation day 1.

Mouse number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Marking												
***I**n /**o**ut of nest*												
Milk spot*												
Weight (g)												
***P**assive/**A**ctive*												
Vocalization when handled (Yes/No)												
Notes												
Signature/date												

* S.k. ”milk spot” you can see a white spot on the pups belly when the pup has suckled the mother .

Appedix 3.

Nest score grading system.

Score	View from above	View from side
1		
2		
3		
4		
5		

Hess et al. 2008

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