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Novel Bedding Material Results in Poor Pregnancy Rate with CD-1 Female Mice Used as Fosters for Producing Transgenic Mice

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Summary

The impact of a novel bedding material (cotton cloth) on the reproductive performance (pregnancy rate and production of offspring) was studied in foster females used for producing transgenic mice. Embryos injected with DNA were transferred to pseudo-pregnant foster females housed under standard conditions (aspen bedding and nesting material). After embryo transfer, mice were divided between the experimental group (AGREBE cotton cloth) and control group (aspen bedding and nesting material). Pregnant mice were observed at day 15 after the transfer and the number of offspring was recorded on post-natal days 3 and 21. Altogether 116 foster mice were used as embryo recipients. Significantly more pregnancies were observed in the control group versus the experimental group: 43% and 19% of foster mice, respectively. Informal interviews with animal caretakers revealed a general dislike towards the cotton cloth (dirtier cages, mice often found on the plastic cage surface, difficult husbandry routines). The cotton cloth showed major signs of wear and tear after only a few weeks of usage. In conclusion, this study with female mice demonstrated that a cotton cloth cannot be recommended as a sole replacement for bedding and nesting material.

Keywords: Mice, bedding, reproduction, welfare

Introduction

The welfare of laboratory mice has become increasingly important in recent years. Animal welfare is not only a requirement enforced by laws and regulations, it is a fundamental part of good scientific practice (*Directive 2010/63/EU)*. An essential part of animal welfare for mice kept in laboratory conditions is how they experience their immediate environment. This micro-environment can be optimised by utilising appropriate temperature, humidity, ventilation, intensity of light and light cycle to match the experimental or husbandry requirements (*Directive 2010/63/EU ; National Research Council, 2011*). Additionally, bedding and nesting material, together with environmental enrichment, play an important role in mouse welfare (*Gaskill et al., 2013; Hess et al., 2008; Jackson et al., 2015*) by providing a suitable physical environment, as well as the environment needed for species-specific behaviors (*Smith & Corrow, 2005; Van de Weerd et al., 1997*) and could have a major impact on how mice perceive their immediate micro-environment.

Many modern animal facilities use Individually Ventilated Cages (IVC) that create an unique micro-environment. The micro-environment with IVC systems relies on active ventilation, resulting in 30 to over 100 air changes per hour (ACH) (*Baumans et al., 2002*). Thus, one could expect that bedding and nesting material with higher insulation ability could potentially be preferred by mice (*Baumans et al., 2002; Gaskill et al., 2013b; Gordon et al., 2014; Jackson et al., 2015*). Optimising bedding and nesting material for both experimental and husbandry needs would be beneficial for both mice and science.

Historically, wood chips and shavings, as well as cellulose granules, have been widely used for both bedding and nesting (*Blom et al., 1996; Van de Weerd et al., 1997*). Additional nesting materials and structures, such as wooden and plastic enclosures, have been developed to support nest building (*Olsson & Dahlborn, 2002*). New materials are constantly sought with the aim of improving mouse welfare and breeding.

By introducing a large cotton cloth (*Kawakami et al., 2007; Kawakami et al., 2012*) into a cage instead of bedding and nesting material we thought it may enhance the welfare of the mice as well as their breeding efficiency. The cloth could enable the mice to build enclosed nests, thus creating a warmer nest that would potentially protect them from IVC-generated draughts. Additionally, the cloth could be reused after washing and sterilizing, thus offering an environmentally conscious alternative to standard bedding and nesting material. Furthermore, using cloth instead of bedding and nesting material could reduce dust in the cages allowing for longer intervals between cleaning and washing of the ventilated racks. Therefore, the aim of this study was to determine if this novel approach using only a large cotton cloth in a cage would support better breeding efficiency and animal welfare. We measured reproductive performance, pregnancy rate and number of offspring produced, as indicators of animal welfare (*Broom & Johnson, 1993*), in a colony of foster mice used to produce transgenic offspring. Additionally, we interviewed animal caretakers for their subjective assessment regarding the practical usability of the cloth and how it fits into husbandry routines.

Material and Methods

Animals and husbandry

Hsd:ICR (CD-1®) in-house bred female mice, 9 – 12 weeks of age, weighing 25-35g, were maintained in pathogen-free conditions (according to FELASA 2002 recommendations (*Nicklas et al., 2002*)) and housed in an IVC system (type 1145T, cage dimensions (W x D x H) 403 x 165 x 174 mm; Tecniplast, Buguggiate, Italy). These mice received embryos from pronuclear injection experiments. Each cage was provided with either 160g of autoclaved aspen bedding and a full handful of nesting material (Tapvei, Paekna, Estonia), or an autoclaved cotton cloth (AGREBE Cotton Cloth, 55cm x 55cm, Duo-Medix OHG, Hamburg, Germany) (Figure 1). Cages were changed once per week under a laminar flow changing station (InterActive Cage Changing Station; Tecniplast, Buguggiate, Italy). Cotton cloths were machine washed (90°C without detergent) and autoclaved weekly, and subsequently reused. Pelleted autoclaved mouse diet (Harlan Teklad 2018S; Harlan, Indianapolis, IN, USA), as well as acidified (pH 3.0) water, was provided *ad libitum*. The room was kept at 22±1°C, with a relative humidity of 55±10%, and a 12-hour light/dark cycle with lights off at 17:00.

All animal housing, handling, and experimental techniques were in accordance with the principles set out in the Declaration of Helsinki, as well as in

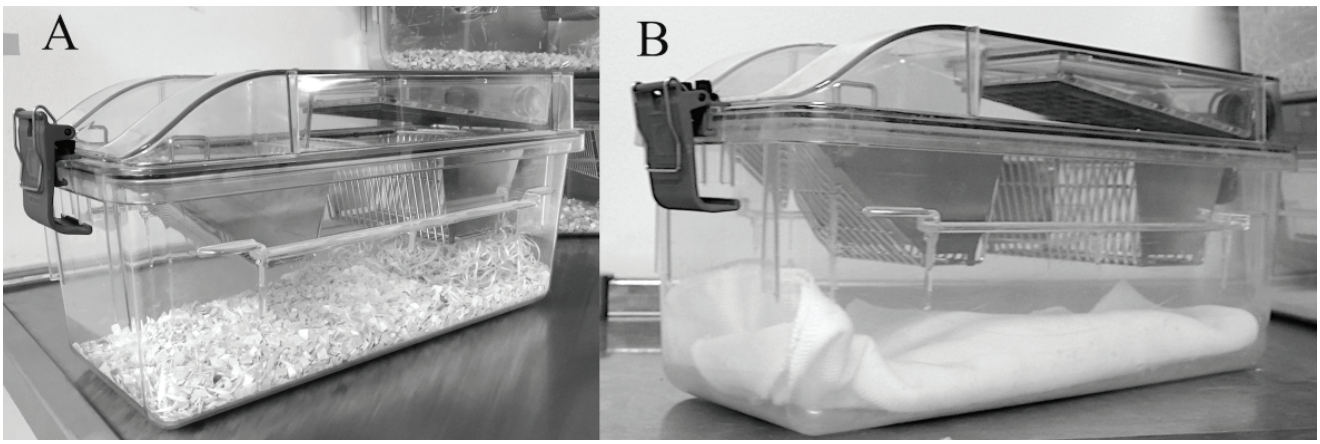

Figure 1. A) Standard cage with aspen bedding and nesting material. B) Experimental cage with AGREBE cotton cloth.

accordance with the ethical standards of the European and German Animal Welfare legislation. Experiments were planned and conducted to adhere closely to the 3R (replacement, reduction, refinement) principles of animal welfare. All procedures were licensed and carried out according to the Institutional Animal Care and Use Committee guidelines as regulated by the German Federal law governing animal welfare.

Experimental design and data collected

The experiment was performed during the standard production of genetically modified mice. In short, embryos injected with DNA were transferred to pseudo-pregnant foster females under general anaesthesia. Foster females were housed under standard conditions (aspen bedding and nest material) until the embryo transfer procedure, after which they were equally divided between the experimental group (cotton cloth) and control group (aspen bedding and nest material). Typically, two foster females were placed in each cage, and they stayed together until weaning. In some rare cases (only if an odd number of foster females were available) a single foster female was placed in a cage. All mice received, on the same day, embryos with the same DNA construct. Altogether 116 foster mice were used as embryo recipients (63 with aspen bedding, 53 with cotton cloth). On average twenty to twenty-five injected embryos were transferred to each foster. Pregnancies were observed (visual verification and palpating) 15 days after the transfer and mice were scored 'pregnant' or 'not pregnant'. On post natal day (PND) 3 the number of offspring was recorded. The offspring were weaned and the numbers of male and female pups were counted on PND21.

Statistics

All statistical analysis was performed using IBM SPSS Statistics version 23 software. The Pearson Chi-Square test was used to compare the groups for the number of females pregnant and the number of females weaning pups. The number of pups born was compared, using the Mann-Whitney U-test, in two ways: for all females and for pregnant females. Male:female ratio at weaning was compared, using the Pearson Chi-Square test, based on pooled data for each of the two groups. The values $p<0.05$ were considered statistically significant.

Results

The summary of results can be seen in Table 1. Significantly more pregnancies were observed in the aspen bedding group as compared to the cotton cloth group. All of the observed pregnancies from both groups produced live offspring. In total, the aspen group produced almost three times the number of pups compared to the cotton cloth group, 87 versus 32, respectively. No pre-weaning loss of pups was recorded in the aspen bedding group, however two pups were lost in the cotton cloth group. Although the yield of offspring (the litter-size) was similar per pregnant female in both groups, the number of offspring born per female was significantly reduced in the cotton cloth group versus the aspen bedding group. Male to female ratio was equal in the aspen bedding group but notably shifted to favour males over females in the cotton cloth group, although the difference between the groups was not statistically significant.

Table 1. The reproductive performance and male:female ratio of pups in the litters of embryo-transferred mice housed with either traditional aspen bedding or with a cotton cloth.

	Aspen Bedding	Cotton Cloth	Statistics	Statistical test
Number of foster females (n)	63	53	-	-
Number of pregnancies	27 (43%)	10 (19%)	$X^2(1)=7.626$, p=0.006	Pearson Chi-Square
Pups born per female	1.38 ± 1.84	0.60 ± 1.47	U=1250.5. p=0.005	Mann Whitney U Test
Pups born per pregnant female	3.22 ± 1.37	3.20 ± 1.81	U=116.5, p=0.533	Mann Whitney U Test
Males weaned	44 (51%)	19 (63%)	$X^2(1)=1.461$, p=0.227	Pearson Chi-Square
Females weaned	43 (49%)	11 (37%)		
Male:Female ratio	1.02	1.73	-	-

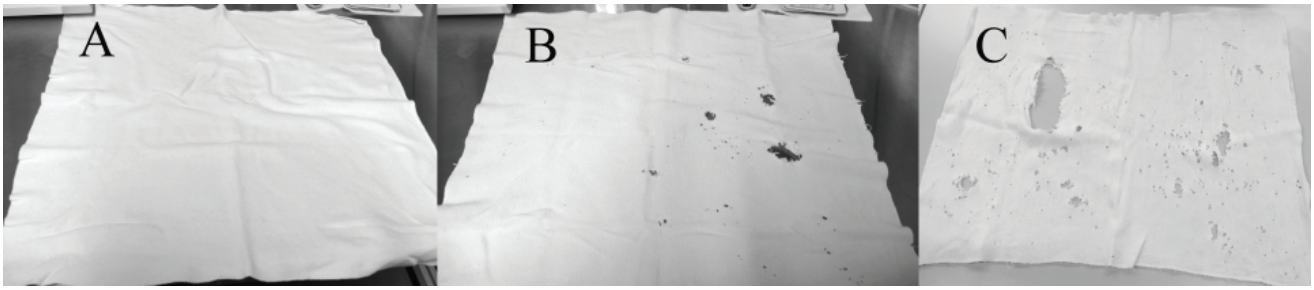

Figure 2. Cotton cloth showed significant amount of wear after only few weeks of usage. A) new unused cloth, B) cloth after one week of use, and C) cloth after three weeks of use.

Discussion

An earlier study (*Kawakami et al., 2007*) showed a clear preference towards cotton cloth when mice were offered free access to three different bedding materials and the cloth. Yet, in our study, breeding performance dramatically deteriorated when mice were housed in a cage with a cotton cloth, demonstrating that a cotton cloth as a sole replacement for bedding and nesting material is not to be recommended. However, the utility of a cotton cloth if other material was accessible as well remains unknown.

It is also worth noting that the cotton cloth in our study was provided as a novelty item for recipients of embryo transfer, which may have been stressful for the females. Interestingly, the increase of male versus female pups in the cotton cloth group was noticeable (although not statistically significant), and could possibly indicate elevated stress levels in the foster mothers. A similar relationship has been shown in squirrels where elevated stress during the gestation period was more likely to produce male-biased litters (*Ryan et al., 2011*).

Informal interviews with animal caretakers revealed that they initially liked the option for mice to be completely covered by the cloth. But more importantly, animal caretakers felt that with a cotton cloth the cage appeared dirtier, mice hidden from view were actually lying under the cloth directly on the plastic cage surface, and when trying to perform husbandry duties mice were more difficult to catch and handle. It has been shown (*Van de Weerd et al., 1997*) that mice, when given a choice, typically would prefer cotton-like material or tissue for building nests. Interestingly, in this study mothers with litters seemed to spend more time lying directly on the plastic surface under the cloth, and did not build elaborate nests from the cotton cloth. The reason why mice selected the bare cage bottom under the cotton cloth was not clear, although it could be speculated that being covered by the cloth nevertheless provided enough nest-like environment even when mice were lying directly on the plastic floor. It is possible that this behaviour could have contributed towards poor reproduction. Furthermore, the cotton cloth wore out quicker than anticipated, and showed major signs of wear and tear (holes of various sizes) after only a few weeks of usage (Figure 2). This was disappointing and undermines the potential positive environmental impact of a reusable cloth.

In conclusion, based on overwhelmingly negative feedback from animal caretakers, combined with poor reproduction results, and the relatively short period of reusability, it was decided not to utilise the cloth material any further as a sole replacement for standard bedding and nesting material. A similar but much smaller cloth may eventually offer additional environmental enrichment when used in combination with standard bedding material, but this is still to be studied.

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