Can accurate control of relative humidity at minimum 55% affect health of research animals, breeding performance and validity of research?

Karen Ekkelund Petersen (kep@scanbur.com) DVM, PhD, Scientific Affairs Manager, SCANBUR A/S

Abstract

EU regulatory guidelines for relative humidity (RH) when housing laboratory rodents are 55 ± 10%. These were set based on quite limited data and little is known on how fluctuations in RH affects laboratory rodents, or how high versus low RH, within these boundaries affects them. Studies through time have shown that RH has an impact on rodent health.

We have set up several studies to investigate the effect of tighter control of RH on health of research animals primarily by investigating the effect on breeding performance. Additionally, the potential of uncontrolled RH to affect outcomes of studies, and thus validity of research, has been part of our studies. We have specifically looked at RH controlled accurately at 55% or above. These studies have shown that a controlled RH improves breeding performance in different setups, and outcome of studies where e.g. water intake is an outcome or thirst a motivator. We are continuously working on additional studies to investigate effects of controlled RH on other research areas.

Introduction

One particularly well-known example of the effects of RH on rodent laboratory animals is connection between low RH, and the presence of ring tail in mice and rats[1,2]. This problem of low RH is well-described, but it is not the only or most important problem with poor control of RH. Low RH has shown to increase activity in mice[3] and home-cage environment including RH levels could potentially affect the outcome of behavioural studies[4,5]. Investigation of how variations in RH within guidelines affect performance in behavioural studies is not yet investigated in detail. Moreover, interesting parameters such as stress and aggression could be observed as well, to give indication of some of the mechanism behind a potential difference in performance in behavioural testing.

The reproducibility crisis of preclinical research has been debated for years and no conclusion has been drawn for its sources yet[6]. Standardization of environmental factors when housing laboratory animals is one of the parameters discussed in this context, and even if it is believed to be the cure for the reproducibility crisis or not, the ability to control environmental factors is crucial in preclinical research[7].

RH has impact on growth conditions for bacteria and fungus and transmission of virus[8-11] and could by these means have direct impact on the health and wellbeing of laboratory animals. Additionally, this could impact research results when these factors are the study outcomes.

Bringing the knowledge from other species on how RH affects reproduction, sows are a good example. In the concept “heat stress” both heat and RH plays a role and it is known that pigs have reduced breeding performance when subjected to high temperature and RH, while piglet survival increases[12,13]. Little is known about the effect of RH alone and in laboratory animals, however it has been shown that puberty is delayed in female mice housed

under 15-30% RH, whereas first estrus was attained earlier when housed under a RH of 75%[14]. Variation in breeding performance during the year is known on a anecdotal basis. The possibility to accurately control RH in IVC cages and cabinets has led the investigation of how tighter controlled RH affects breeding of rodents.

Problem statement

Limited data are available on how accurate humidity within guidelines affects animal welfare and breeding performance of various animal models. We assume that these were made to avoid harm and suffering of research animals taking the performance of existing technology in to account. With the technology that now exist we can accurately control RH and with the data that has been generated on an ongoing basis it is emphasized that RH can affect animal welfare, performance of breeding and even study results. These data have been the starting point of the study design we have made.

Solution

Until recent years the control of RH has been difficult to manage accurately in animal facilities, and the control has been made on room level. Newer technology makes control of RH at cage level possible, allowing for very precise control of the humidity (ScanClime, SCANBUR A/S).

Anecdotal feedback and preliminary findings from various research institutions indicate that controlling RH, especially avoiding the low RH levels seen in the wintertime, when outside temperatures drop, can impact breeding in mice and rats, embryo transfer success rate, skin health and aggression of male mice. The data are continuously being collected and will be published ongoing. Literature and preliminary data support that RH has an impact on animals. How can this have impact on study results, animal welfare and even reproducibility of research? We have performed studies in collaboration with different research facilities and found the following:

<u>Study in collaboration with University College London, United Kingdom[15]:</u>

• Findings:

- Controlling RH at 45%, 55% or 65% led to mice drinking significantly less than mice housed under housing conditions with RH not being tightly controlled.

<u>Study performed at the University of Turku, Finland[16]:</u>

• Findings:

- Pre-weaning mortality was significantly lower in mice housed under tighter controlled RH during a whole calendar year (16% vs. 23%). Similarly, pre-weaning mortality was lower during low humidity period (winter) in mice housed at controlled RH (22% vs. 32%).
- This shows that reproductive performance is affected by RH.
- It is possible that factors other than RH might influence the pre-weaning mortality as well.

Study in collaboration with King's College London, United Kingdom[17]:

• Findings:

- The number of pups per litter was significantly higher for animals housed under tighter controlled RH conditions (↑ 12%). The same was found when only investigating mice with various C57BL/6 backgrounds (↑ 10%).
- Total litter loss registered at weaning was significantly higher in the group housed under less controlled RH conditions (↑ 30%).
- No significant difference was found in preweaning mortality.
- Variation in RH in the first week postpartum was correlated to total litter loss, showing that high variation was associated with higher percentage total litter loss and low variation with a low percentage of total litter loss.

Additionally, preliminary findings from a study on breeding performance suggest a strain variation on how much tighter control of RH improves breeding performance. One of hypotheses is that the effect on breeding could be most pronounced in immunodeficient and nude mice. This is currently investigated in a study at a large breeding facility. Here the same strain is used, bred in the same room.

General limitations to the studies: The system used for controlling of humidity in most of the studies, ongoing and completed, can only humidify, thus the RH is set to e.g. minimum 55% but could go above during periods with high humidity. The breeding data is collected from animals housed in different rooms and collected on various strains.

Conclusions:

The literature and the results we have found support that RH affects study results, animal health and our results show that tighter control of RH can improve murine breeding performance significantly. Further investigation is warranted on how different levels of stable RH versus variation in humidity affect the breeding performance. Studies are performed ongoing to further investigate the effect of RH on breeding performance, embryo transfer success rate, aggression of male mice, skin health etc. The anecdotal feedback from existing installations is investigated further to accept or reject hypotheses of effects of RH on animals and research results.

Are you interested in more details? Several studies and a comprehensive list of literature is available when visiting SCIENTIFIC at www.scanclime.com

References:

1 Ellison, G. T. et Westlin-van Aarde, L. M. Ringtail in the pouched mouse (Saccostomus campestris). *Lab Anim* **24**, 205-206, doi:10.1258/002367790780866209 (1990).

2 Totton, M. Ringtail in new-born Norway rats; a study of the effect of environmental temperature and humidity on incidence. *The Journal of hygiene* **56**, 190-196, doi:10.1017/s0022172400037682 (1958).

3 Clough, G. Environmental effects on animals used in biomedical research. *Biological reviews of the Cambridge Philosophical Society* **57**, 487-523 (1982).

4 Tang, X., Orchard, S. M. et Sanford, L. D. Home cage activity and behavioral performance in inbred and hybrid mice. *Behavioural brain research* **136**, 555-569, doi:10.1016/s0166-4328(02)00228-0 (2002).

5 Chesler, E. J., Wilson, S. G., Lariviere, W. R., Rodriguez-Zas, S. L. et Mogil, J. S. Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. *Neuroscience & Biobehavioral Reviews* **26**, 907-923, doi:https://doi.org/10.1016/S0149-7634(02)00103-3 (2002).

6 Begley, C. G. et Ioannidis, J. P. Reproducibility in science: improving the standard for basic and preclinical research. *Circulation research* **116**, 116-126, doi:10.1161/circresaha.114.303819 (2015).

7 Richter, S. H., Garner, J. P. et Würbel, H. Environmental standardization: cure or cause of poor reproducibility in animal experiments? *Nature methods* **6**, 257-261, doi:10.1038/nmeth.1312 (2009).

8 Arundel, A. V., Sterling, E. M., Biggin, J. H. et Sterling, T. D. Indirect health effects of relative humidity in indoor environments. *Environ Health Perspect* **65**, 351-361, doi:10.1289/ehp.8665351 (1986).

9 Alsmo, T. et Alsmo, C. Ventilation and Relative Humidity in Swedish Buildings. *Journal of Environmental Protection* **05**, 1022-1036, doi:10.4236/jep.2014.511102 (2014).

10 Lowen, A. C., Mubareka, S., Steel, J. et Palese, P. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS pathogens* **3**, 1470-1476, doi:10.1371/journal.ppat.0030151 (2007).

11 Van Der Veen, J., Poort, Y. et Birchfield, D. J. Effect of Relative Humidity on Experimental Transmission of Sendai Virus in Mice. *Proceedings of the Society for Experimental Biology and Medicine* **140**, 1437-1440, doi:10.3181/00379727-140-36691 (1972).

12 Wegner, K., Lambertz, C., Das, G., Reiner, G. et Gauly, M. Climatic effects on sow fertility and piglet survival under influence of a moderate climate. *Animal* **8**, 1526-1533, doi:10.1017/s1751731114001219 (2014).

13 Suriyasomboon, A., Lundeheim, N., Kunavongkrit, A. et Einarsson, S. Effect of temperature and humidity on reproductive performance of crossbred sows in Thailand. *Theriogenology* **65**, 606-628, doi:10.1016/j.theriogenology.2005.06.005 (2006).

14 Drickamer, L. C. Environmental factors and age of puberty in female house mice. *Developmental psychobiology* **23**, 63-73, doi:10.1002/dev.420230107 (1990).

15 Petersen, K. E., Towns, R., Andersen, C. H. et Sunesen, M. The effect of relative humidity on water intake of C57BL/6J mice housed under conditions of controlled relative humidity at cage level (2018).

16 Major, M. M. *et al.* Pre-weaning mortality of mice housed in individually ventilated cages with and without controlled humidity (Central Animal Laboratory, University of Turku (UTUCAL), Turku, Finland, 2019).

17 Petersen, K. E., Ringsted, A. et Woodley, S. Tighter control of relative humidity improves murine breeding performance – a retrospective analysis (2019).