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Effects of an Extended Cage-change Interval on Ammonia Levels and Reproduction in Mongolian Gerbils (*Meriones unguiculatus*)

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Prompted by the cage cleanliness of Mongolian gerbils (*Meriones unguiculatus*), we evaluated a prolonged cage-change interval. We compared the effects of a 2-wk and 6-wk cage-change schedule on ammonia levels, temperature, humidity, and reproductive performance in breeding pairs housed in IVC. We hypothesized that ammonia levels would remain below our threshold for cage changing and that reproductive performance would not be affected. Although ammonia levels increased over time, they remained low (less than 5 ppm) over the 6-wk period. In addition, the 6-wk cage-change interval did not significantly influence reproductive parameters, such as average pup weaning weight, number of litters, and number of pups per litter. We conclude that an extended cage-change interval (6-wk) can be used for gerbils without significant increases in intracage ammonia levels or effects on reproduction.

At the University of Colorado Denver Anschutz Medical Campus, Mongolian gerbils (*Meriones unguiculatus*) are housed at a variety of densities, varying from single housing for an experimental study to breeding pairs with litters, for a maximum of 8 pups and 2 adult animals. We noted that IVC-housed gerbils were very clean when they were changed every 2 wk, even for breeding pairs with many weaning-age pups. Qualitatively, the cages had dry bedding and minimal fecal loads with little ammonia odor. Furthermore, the natural habitat of gerbils in eastern Mongolia is a generally very arid environment that includes deserts; therefore, gerbils have adapted to a lack of water by secreting scant concentrated urine.[7,11] In comparison to other common laboratory rodents, gerbils may be an ideal species for extended cage-change intervals because of their low urine production. For example, gerbils produce 2 to 4 drops (1 drop = 0.05 mL) of urine per 24 h, whereas a much smaller mouse may produce 0.5 to 2.5 mL of urine in 24 h.[12] In a laboratory environment, where water is plentiful, whether gerbils still have low urine production is unknown. Although we did not examine this question specifically, we believe that urine production of gerbils would be less than that of other common rodents because gerbils are adapted to living in arid environments. Related to this, gerbils used in research originated from their wild counterparts in 1935.[11]

Because of their potentially low urine production, we assessed whether gerbils housed in IVC could be changed less frequently than every 2 wk. Less frequent changing is ideal because it reduces labor and costs, and it minimizes stress on the animals.[14] Subsequently, these savings could translate to lower per-diem costs that can be passed on to investigators.

Numerous previous publications have assessed cage-change frequency and its effect on animal welfare in rodents, especially given the increased use of IVC.[1,3,8,10,16-17,19,23,24] However, to our knowledge, the current report is the first publication to examined an extended cage change interval in gerbils. We chose to test the extended cage-change interval on our breeding pair colony for several reasons. First, these animals could be housed stably long-term for this study without disrupting other ongoing research projects. Second, the combination of a breeding pair and weaning age litter is one of our highest density housing set-ups, and thus ammonia production should be highest in these cages. Third, the gerbils were breeding well at the cage-change interval of 2 wk, and we wanted to ensure that a longer cage-changing interval would not have a detrimental effect on breeder performance. We hypothesized that breeding performance would not be affected by a longer cage-change interval. We also hypothesized that, due to the scant urine production, ammonia levels would remain low enough for cages to be changed less often than every 2 wk and tested environmental parameters up to 6 wk.

Materials and Methods

Animals. In this study, we used 10 breeding pairs of gerbils, which ranged from 259 to 482 d old at the start of the study. Ages were matched between groups to control for age effects on breeding performance. Animals originally were obtained from Charles River Laboratories (Wilmington, MA) in 2010 and have been maintained as a breeding colony, with intermittent introduction of new animals from Charles River Laboratories to prevent genetic drift. Gerbils were tested quarterly for parasites by fecal floatation, perianal tape test, and fur plucks, but no serologic testing was performed. All animals were negative for any parasites tested. All gerbils were on an IACUC-approved protocol for another study.

Housing. Gerbils were housed in MultiSpecies cages (catalog no. PCT4SHT, Allentown Caging, Allentown, NJ) that are 15 in. × 19.5 in. × 8.5 in., corresponding to 210 in.^{2} of interior floor space. Animals were housed on aspen bedding (catalog no. 7090A, Teklad Aspen Sani-chips, Envigo, Huntingdon, Cambridgeshire, United Kingdom), with a plastic shelter as enrichment. Animals were fed a commercial rodent diet (Teklad 2920X, Envigo) and

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received chlorinated water through an automated watering system (Edstrom Industries, Waterford, WI). All cages were on a single-sided 18-cage ventilated rack (Microvent, Allentown) distributed among 4 rows. The rack was not replaced during the study. A rack flow detector (Allentown) measured an air supply rate of 26.1 ft^3 per minute, which according to the manufacturer, corresponds to 58 ± 5 air changes hourly. The rack air flow measurement was repeated midway through the study to confirm consistent air flow. Numbers of pups and litters were recorded as soon as a litter was noted. Body weights were measured at weaning, which ranged from d 29 to 31. All animals were housed in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*[9] and all federal regulations.

Measurements. We monitored 5 cages per group over a 12-wk period. From June through August, one group underwent 6 cage changes at 2-wk intervals whereas the other group received 2 cage changes at a 6-wk interval. Temperature and humidity were recorded (model RH520A, Humidity and Temperature Recorder, Extech, Nashua, NH). Cages were slid forward a few inches off the rack so that lids could be lifted approximately 1 in. to place the detector into the cage to measure the temperature and humidity at approximately 1 in. above the cage floor. The detector was left in the cage for a minimum of 1 min to allow temperature and humidity readings to stabilize.

To measure ammonia levels, a gas-aspirating pump (model AP-20, Kitagawa America, Pompton Lakes, NJ) with ammonia gas detector tubes (catalog no. 105SD, 0.2 to 20 ppm, Matheson–Kitagawa, Montgomeryville, PA) was used. The cage was removed from the rack, turned 180°, and placed backward into the rack. The grommet for the automatic watering system valve was used to position the ammonia gas detector tube in the cage approximately 1 in. above the cage floor. According to the manufacturer's instructions, the pump was pulled to a full stroke, locked position, and a color change indicated the ammonia level on the tube. If no color change occurred on the initial reading, the full stroke was repeated until 5 times total and the resulting reading was divided by the number of strokes needed to achieve the minimal detectable limit of 0.2 ppm for these tubes. As indicated by the manufacturer, no corrections for variations in temperature or humidity are required for ammonia measurements. A correction factor of 1.21× was applied to the tube reading to account for Denver's atmospheric pressure of 834 hectopascal. The manufacturer reports that the tubes have a relative standard deviation of 10% at low readings and 5% at mid-range and high readings. Once we established that ammonia levels were less than 0.2 ppm in a clean cage on day 0, further measurements were not performed in new cages after changing.

Pathology. To assess potential pathologic effects associated with increased ammonia levels, nasal histopathology was examined. At the end of the study, 2 gerbils that were to be retired as breeders were selected from each group and euthanized (100 mg/kg IP, Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI). Tissues were preserved in 10% formalin and decalcified for 3 wk, with multiple changes of freshly made 10% EDTA–7% glycerol solution. Tissues were paraffin-embedded and sectioned at 4 µm. Tissue slides were stained with hematoxylin and eosin.

Statistical analysis. Reproductive variables including average pup weight at weaning, number of litters, and number of pups per litter were examined by comparing their distributions in the 2 treatment groups—less frequent changing (that is, every 6 wk) compared with control (that is, every 2 wk)—by using a standard 2-tailed *t* test (version 7, Prism for Mac, GraphPad Software, La Jolla, CA). For variables such as cage temperature, humidity, and ammonia levels, a mixed-effects model was used to account for correlations due to repeated observations on the same cage or litter; treatment group and time elapsed since the last cage change were incorporated into the model as fixed effects (R program; https://www.R-project.org/).[13] A P value of less than 0.05 was considered significant.

Results

The baseline temperature in all cages increased by approximately 0.11 ± 0.03 °F per week over 12 wk. This effect occurred in both groups and was presumably due to reasons unrelated to the study. More importantly, for each week that the cages were not changed, an additional increase of 0.44 ± 0.19 °F occurred for the 2-wk cage-change condition and 0.05 ± 0.07 °F for the 6-wk cage change condition. This difference in weekly increases is equivalent to 2.4 SD, which is statistically significant according to conventional standards. During each week that the cages were changed, the temperature reverted to baseline (Figure 1 A).

The baseline humidity in both groups increased by approximately 0.27% ± 0.07% per week over 12 wk. For each week that the cages were not changed, an additional increase of 1.31% ± 0.41% was observed for the 2-wk cage-change condition and 0.23% ± 0.15% for the 6-wk cage-change condition. This difference in weekly increases represents 3.3 SD, which is statistically significant by conventional standards. In each week that the cages were changed, the level reverted back to baseline (Figure 1 B).

Ammonia levels in both groups increased by approximately 0.10 ± 0.03 ppm per week over 12 wk. For each week that the cages were not changed, an additional increase of 0.40 ± 0.23 ppm occurred for the 2-wk cage-change condition and 0.41± 0.08 ppm for the 6-wk cage-change condition. This difference is less than 1 SD and thus statistically negligible. The accumulation lasted for 6 wk in experimental cages but for only 2 wk in control cages. During each week that the cages were changed, the ammonia level reverted back to the slowly increasing baseline (Figure 1 C).

Average pup weight at weaning between groups (2-wk group, 19.5 ± 1.2 grams, 6-wk group, 20.7 ± 1.4 grams, P = 0.5637, Figure 2 A), number of litters per group (2-wk group 2.6 ± 0.7 litters, 6-wk group, 2.4 ± 0.2 litters P = 0.7885, Figure 2 B), and the number of pups per litter (2-wk group, 4.25 ± 0.64 pups, 6-wk group, 4.23 ± 0.57 pups, P = 0.9822, Figure 2 C) did not differ significantly between groups.

Nasal histopathology did not reveal any abnormalities (Figure 3). The ciliated respiratory epithelium was normal, as were the mucosal glands, teeth, nasal septum, and vasculature, including lymphatics.

Discussion

In this study, we compared a 2-wk cage-change interval with a 6-wk interval to assess potential effects of these intervals on the health and reproductive performance of gerbils. Concerns regarding a longer change interval include ammonia accumulation and associated negative health effects. However, as we hypothesized, the ammonia levels in all gerbil cages, including those changed at 6-wk intervals, remained significantly lower than our cage-change threshold (50 ppm), indicating that cages could be changed less frequently than every 2 wk. Numerous publications report levels of ammonia that might negatively affect animal health, but a consensus threshold has not been

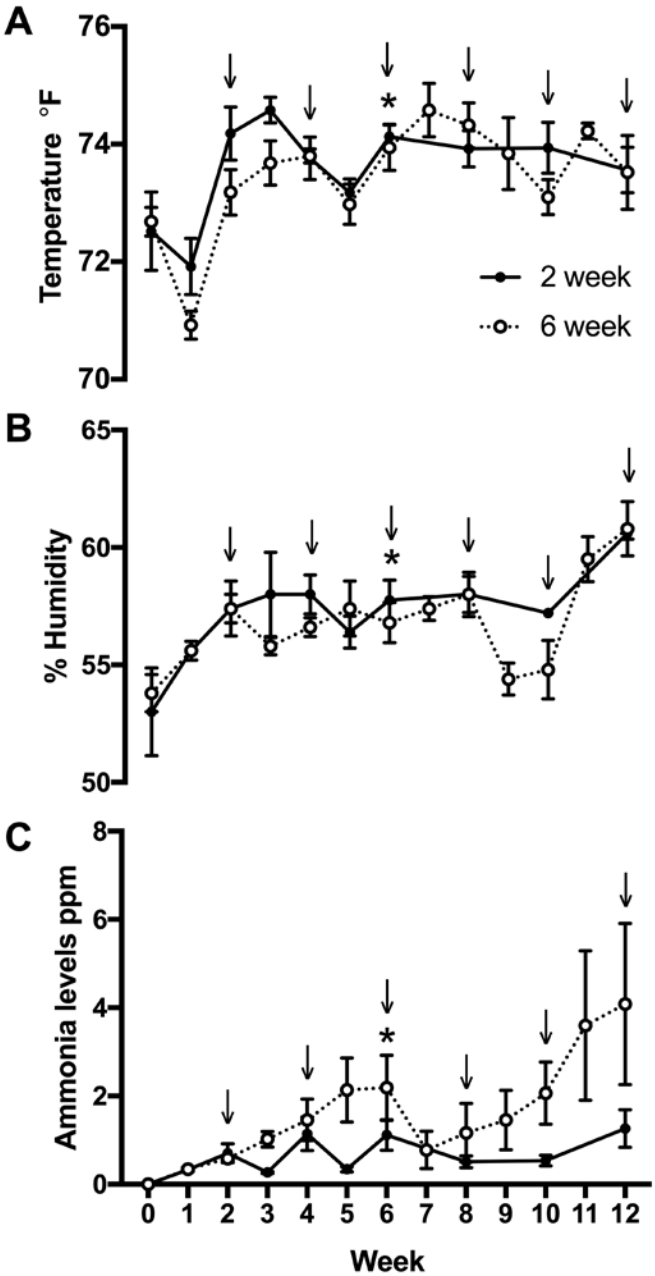

Figure 1. (A) Temperature, (B) humidity, and (C) ammonia levels for the 2-wk (solid circles) and 6-wk (open circles) cage-change intervals. Weeks in which cages were changed after ammonia was measured are marked by an arrow for the 2-wk group and an asterisk for the 6-wk group.

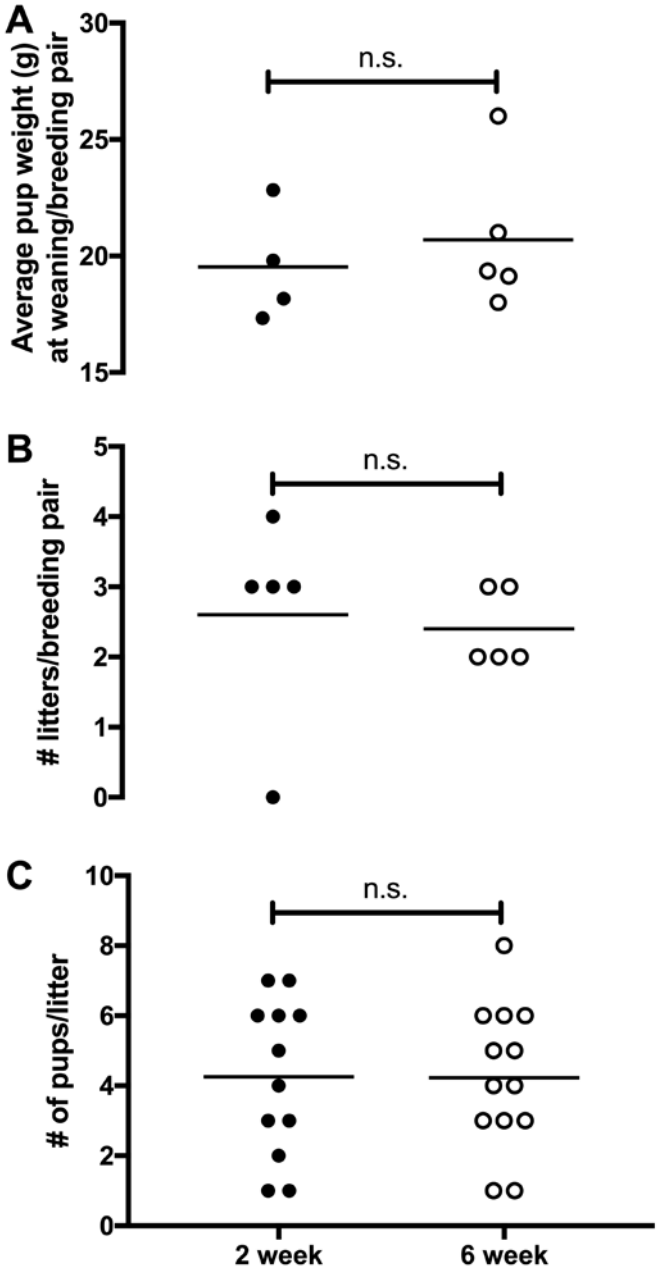

Figure 2. Reproductive parameters for gerbil cage change groups during the total 12 wk of study. (A) Average pup weight (g) at weaning. (B) Number of litters. (C) Number of pups per litter. Solid circles, 2-wk cage-change group; open circles, 6-wk cage-change group; n.s., not significant.

established.[3-6,10,16,19,23] For example, one study reported that although no ammonia level is absolutely safe, the observation of histologic lesions suggested that ammonia should remain below 25 ppm.[23] However, another study found no histopathology associated with various ammonia levels above 100 ppm.[22] Given the range of values in the literature, we used 50 ppm as a midlevel threshold. The ammonia levels remained low, less than 5 ppm even at 6 wk, and thus more than 10fold below our threshold. Even with a threshold of 25 ppm, which has been commonly discussed,[4,8,16,17,20,23,24] the ammonia levels were still well below this value. Although histopathologic lesions would not have been expected at the low ammonia levels that were measured, we still performed nasal histopathology to assess for complications associated with elevated ammonia. Due to the unlikelihood of observing lesions, only 2 gerbils from each group, which were to be retired as breeders, were submitted for nasal histopathology at the conclusion of the study. All nasal histopathology was normal.

Temperature and humidity remained relatively stable throughout the two 6-wk monitoring periods. The weekly increases in temperature and humidity of the 2-wk group (0.44 ± 0.19 °F, 1.31% ± 0.41%) were significantly greater than those of the 6-wk group (0.05 ± 0.07 °F, 0.23% ± 0.15%). The reasons underlying this difference are unknown, but most importantly, over the 6-wk periods, the magnitude of the absolute changes in temperature and humidity were still quite small. For the 6-wk cage-change group, the average temperature and humidity values at the start (72.6 °F, 53%) and end (73.2 °F, 58%) of the

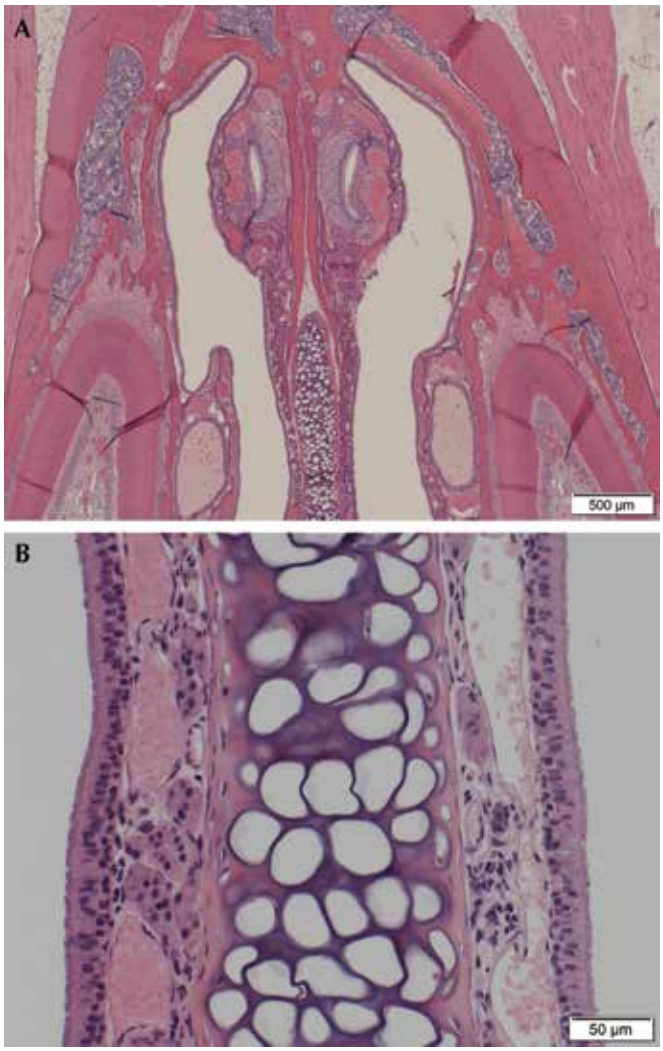

Figure 3. Representative nasal histopathology, without any significant abnormal findings. Magnification: (A) 40× (scale bar, 500 µm) and (B) 400× (scale bar, 50 µm).

study were well within the ranges (68 to 79 °F, /30% to 70%) recommended by the *Guide for the Care and Use of Laboratory Animals*.[9] This pattern was also true for the 2-wk cage-change group. In light of previous reports of low CO_2 levels with extended cage-change intervals and the lack of an evidence-based CO_2 threshold, we chose not to monitor CO_2.[2,3,16,15,19-21]

Reproductive performance did not differ significantly between gerbil cages changed every 2 wk compared with 6 wk. This finding is consistent with studies in mice, for which the frequency of cage changes did not affect breeding performance.[18] If any effect was anticipated, one might expect the longer cage-change interval to slightly improve reproductive performance due to the reduced frequency of cage changes and thus reduced animal stress[14,16] and more productive breeding. However, our main concern was to ensure that a historically successful gerbil breeding colony was not negatively affected by the longer cage-change interval, as was validated in this study. A single breeding pair in the 2-wk cage-change group did not have any litters in the 12-wk period that we measured reproductive output. However, these animals were one of the older breeding pairs (that is, older than 1.5 y), and reduced breeding success is not uncommon at this age. If this data point is removed, the difference between the 2 groups is significant (P = 0.0474), suggesting that the 2-wk cage condition had more litters. However, when the age-matched breeding pair is removed from the 6-wk group, the data no longer differ significantly (P = 0.0972). In addition, the age-matched breeding pair from the 6-wk group had no pups survive from its 2 litters, indicating that age was the main factor contributing to the group without litters. Because pups occasionally were used experimentally prior to weaning, the metric of number of pups successfully weaned could not be compared between groups.

Limitations of the study included the accuracy of the ammonia pump and occasional opening of cages. According to the manufacturer, the manual pump has a relative standard deviation of 10% at low readings and 5% at midrange and high readings. Although the levels of readings are not defined, we presume our readings would be considered low and have a relative standard deviation of 10%. However, even if our readings underestimated ammonia by 3 SD (that is, 30%), the average ammonia level at the 6-wk cage change interval (2.58 ppm) still would have been far less than 25 ppm. Although cages were opened minimally to prevent unequal dispersal of ammonia, this study was performed concurrently with ongoing research that required collecting animals occasionally and thus opening of the cages. In addition, all cages were opened briefly once each week to provide treats and count pups. These limitations may have affected ammonia levels, but we believe that the effect was minimal and would not change our conclusions. One reason for this assumption is that the air exchange rates of IVC are already relatively high such that occasional opening of the cage would have minimal effects. Furthermore, because the ammonia levels in the current study were 10fold less than the threshold prompting cage change, we believe that the main conclusions from these measurements are appropriate even with the caveats discussed. Finally, these limitations are representative of 'real-world' handling of gerbil cages in an animal facility and, therefore, provide a more accurate picture of ammonia levels than would an entirely unhandled cage.

Because of this study, we implemented a 4-wk cage-change interval for all breeding-pair gerbils. We also performed ammonia testing on other housing configurations (lower density but different number, sex, and age) to ensure they could be changed at 4-wk intervals as well (data not shown). We did not extend the cage-change interval to 6 wk initially, because although ammonia is the primary factor to determine when a cage needs to be changed, we wanted to closely monitor other factors involved, such as fecal load. However, the low ammonia levels indicate that the cage-change interval could likely be extended to 6 wk or potentially even further, given that longer time points were not examined in this study. Ammonia levels are clearly dependent on multiple variables, including ventilation, volume, bedding, and air change rate, and therefore should be examined in each specific scenario. However, given our results and the nature of gerbils to potentially produce scant amounts of urine, our findings may encourage others to examine their cage-change interval for gerbils, which have not been reported previously to our knowledge. Longer cage-change intervals are beneficial for the animals by reducing the likelihood of stress, and for the institution by reducing labor, supplies, and costs.

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