Comparative Medicine
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 67, No 5
October 2017
Pages 430–435

*Case Study*

# Sequelae of Occult Aggression Disqualifying Young, Socially Housed, Female New Zealand White Rabbits (*Oryctolagus cuniculus*) from Participation in Dermal Toxicology Studies

**Jeffrey D Wyatt,[1,*] Diane M Moorman-White,[1] Donnalee Ventura,[2] Brett W Schneider,[2] and Thomas W Bittner[2]**

**International animal welfare organizations and federal, regional, and institutional oversight bodies encourage social housing of gregarious species, such as New Zealand white rabbits (*Oryctolagus cuniculus*), to promote animal wellbeing in research, teaching, testing and farming settings. At our institution, 2 groups of female New Zealand white rabbits (approximate age, 11 wk; mean weight, 2.35 kg), compatibly paired at the vendor for 5 wk, were paired in caging or group-housed in a floor pen. The rabbits appeared compatible, demonstrating primarily affiliative behaviors throughout 6 wk of daily observations. However, occult aggression that occurred between daily observations or nocturnally resulted in skin wounding. The skin injuries, first identified during prestudy clipping of fur from the back of each rabbit 6 wk after arrival, disqualified every animal from participation in skin toxicology and muscle implantation studies. Success meeting scientific research requirements while promoting animal welfare and health when socially housing New Zealand white rabbits requires examining the behavioral repertoire of their wild counterparts, European rabbits. Factors including age, sex, and housing density influence territoriality, dominance hierarchy, social ranking, and natural, agonistic, injurious, behavioral tendencies. IACUC and other relevant oversight bodies, researchers, and animal care staff should consider this case study and the species-specific natural history of New Zealand white rabbits when assessing the harm and benefit of social housing in regard to research utility and animal welfare.**

Rabbits (*Oryctolagus cuniculus*) are considered a social species, situationally demonstrating affiliative behaviors including grooming, nuzzling and grazing together as well as agonistic, injurious behaviors under natural and captive conditions.[1,2,5,8,13,15-18,20,21,23-25,30-33] Animal welfare oversight bodies (for example, IACUC), international guidelines (for example, ILAR *Guide for the Care and Use of Laboratory Animals*, European Union Directive on the Protection of Animals used for Scientific Purposes), and AAALAC, International encourage social housing of rabbits to promote a good state of research animal welfare and high quality science.[6,7,12] Therefore rabbits should be assessed in regard to social housing in research, teaching, testing, and farming settings to promote animal wellbeing.

## Case Study

Female New Zealand white rabbits ($n$ = 12; age, 11 wk; mean weight, 2.35 kg) were acquired as 2 groups of 6 rabbits each for use in serial pyrogen, dermal, and muscle implant toxicology studies. The rabbits had been reared together as compatible weanling pairs at the vendor for 5 wk. On arrival at the facility, the rabbits were housed either as a group of 6 in a pen or as the original pairs in double-wide caging. These 2 social housing scenarios were selected because they were the most feasible in our research setting, and this trial was initiated as the institution's first attempt to evaluate social housing of young, female rabbits. All 12 of these rabbits, regardless of social housing style, experienced multiple raking dermal, back wounds over 6 wk, thus disqualifying all of them from enrollment in contract toxicology studies. The incidence of wounding lesions in this case study suggests that balanced risk assessment regarding potential welfare- and research-specific beneficial and adverse consequences of social housing of juvenile, female, New Zealand white rabbits is warranted.

## Materials and Methods

**Oversight body.** The IACUC of the AAALAC-accredited contract research organization performing the toxicology studies approved all animal research facilities and activities, using the *Guide for the Care and Use of Laboratory Animals* as a primary reference resource.[12]

**Animals.** Female New Zealand white rabbits (Crl:KBL(NZW); $n$ = 12; age, 11 to 12 wk; mean weight, 2.35 kg; SPF for reovirus, lymphocytic choriomeningitis virus, parainfluenza types 1 and 2, rotavirus, rabbit hemorrhagic disease virus, bacterial pathogens, and parasites), weaned at 5 wk of age and pair-housed as weanlings with a littermate for approximately 6 wk before shipment, were acquired from an AAALAC-accredited vendor (Charles River, Wilmington, MA). The established pairs were shipped by the vendor in separate compartments of the same crate. Rabbits were individually identified by an ear tattoo placed by the vendor; in addition, on arrival, the fur behind the base of the ears was marked with a unique color from

*Received: 06 Jan 2017. Revision requested: 17 Feb 2017. Accepted: 15 Mar 2017.*
*[1] Division of Comparative Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York, and [2]Toxicology Division, Iuvo Bioscience, Rush, New York.*
*[*]Corresponding author. Email: jeff_wyatt@urmc.rochester.edu*

a permanent marker, as done in other studies of behavior in group-housed rabbits.[5,25] The mean body weight of 41 single-housed, age- and vendor-matched, female, New Zealand white rabbits in caging 18 in. high and providing a total of 5.44 $ft^2$ of floor space (Euro Rabbit Housing, Allentown Caging, Allentown, PA) at our facility was used for body weight comparison with the case study groups.

***Pen-housed rabbits.*** Immediately on arrival, 3 established, compatible pairs of 77-d-old rabbits were socially housed together in a 90-$ft^2$ floor pen at a stocking density of 1 rabbit per 15 $ft^2$ (1.39 $m^2$) for 6 wk. The group size of 6 was chosen given that captive rabbits prefer small social groups (3 to 8 animals), in which they demonstrate a wide range of affiliative, natural behaviors including hopping and playing.[25,33] The front of the pen was constructed with 5-ft high, folding panels of open-wire fencing (slots of 2 in. wide × 10 in. high slots). The remaining perimeter of the pen was accomplished with the animal room's sealed, epoxy-painted walls. The pen was enhanced with a 2-in. layer of aspen–pine hardwood bedding over the epoxy-sealed concrete floor. Additional enrichment included 6 PVC tubes (6 in. wide × 2.5 ft long), plastic hay feeder balls (8 × 8 in.), PVC tubes (diameter, 3 in.; length, 6 in.) and a raised plastic platform (28 × 28 in.; 12 in. off the floor), providing a partially enclosed space. In addition, 6 clear, red plastic shelters large enough to comfortably accommodate 2 rabbits were available. Municipal water was provided without restriction by an automatic watering system (3 valves each on opposite walls of the pen [6 valves total]), with valves mounted 12 in. above the floor and 20 in. apart. Each of the 6 rabbits was provided 125 g of rabbit chow (HFR 5326, Purina Mills International, St Louis, MO) daily in an individual bowl, and the 6 bowls were distributed throughout the pen. Alfalfa hay was provided without restriction in stainless steel receptacles on the pen floor and inserted in the pen fencing. Stainless steel trays (25 in. wide × 27 in. long × 3 in. high) or plastic triangular pans (16 × 16 ×16 in.) containing paper bedding pellets (Cell-Sorb, PeeWee Pet Products, Stuart, FL) were placed in 3 corners of the pen as litter pans to promote the natural behavior of latrine use.[27] The pen was spot-cleaned daily and sanitized every 2 wk; rabbits were moved to a different area outside the pen during cleaning.

***Pair-housed rabbits.*** On arrival, 3 established, compatible pairs of 83-d-old rabbits were re-paired for 6 wk in 2 laboratory cages (Euro Rabbit Housing, Allentown) providing a total of 10.89 $ft^2$ of floor space with a width of 56 in., depth of 28 in., and height of 18 in. and modified with an 8 × 8 in. interconnecting passageway. Each interconnected single cage provided 5.44 $ft^2$ (0.51 $m^2$) of floor space. The double-wide cages had molded plastic bases and were enriched with stainless steel washer rattles (Bio-Serv, Flemington, NJ) attached to stainless steel chains mounted on the cage front and 2 PVC tubes (diameter, 3 in.; length, 6 in.; Figure 1). Water was provided without restriction by an automated water system, with a valve mounted 12 in. above the bottom of each connected cage. Rabbit chow was fed (125 g per rabbit; HFR 5326, Purina) in 2 hoppers and was supplemented with alfalfa hay. Cage pans were emptied each weekday, and cages were sanitized at 2-wk intervals.

***Weighing.*** All rabbits were weighed on arrival. Pen-housed rabbits were weighed weekly. Rabbits paired in cages were weighed 6 wk after arrival, in preparation for enrollment in their first study.

**Husbandry.** ***Housing density.*** The floor space provided to each rabbit in the current study exceeded international standards for research rabbits as well as accepted practices in the meat industry (Figure 2).

**Figure 1.** One side of caging for paired rabbits.

| | Floor space | |
|---|---|---|
| | in $m^2$ | in $ft^2$ |
| Current study: pen (6 rabbits) | 1.39 | 15 |
| Current study: cage (2 rabbits) | 0.51 | 5.4 |
| ILAR *Guide* (< 4 kg)[12] | 0.28 | 3 |
| USDA AWA Regulations (2–4 kg)[3] | 0.28 | 3 |
| EU Directive (< 3 kg)[7] | 0.35 | 3.8 |
| EU Directive (3–5 kg)[7] | 0.42 | 4.5 |
| Meat industry: fattening phase[13,24,31,33] | 0.06–0.07 | 0.65–0.75 |

**Figure 2.** Various guidelines regarding floor space per rabbit.

***Lighting.*** The pen-housed rabbits were maintained on a 15:9-h light:dark cycle to replicate the spring season when wild European rabbits in Australia display primarily affiliative behaviors.[16] The rabbits paired in cages experienced a standard, 12:12-h, light:dark cycle because they shared a room with single-housed rabbits in cages enrolled in other studies.

***Heating, ventilation, and air conditioning.*** Room temperature was maintained at 61 to 72 °F (16.1 to 22.2 °C), with 30% to 70% relative humidity and 100% fresh, nonrecirculating air at a minimum of 10 air-changes hourly.

***Observations.*** Rabbits were observed for 10 to 15 min each morning during provision of husbandry services and monitoring of animal health and wellbeing. Incidents of agonistic and affiliative behavior, described elsewhere[21,25,28] were recorded and tabulated during daily observation periods. Offensive agonistic behaviors included attacking, biting, chasing, vocalizing and thumping. Defensive agonistic behaviors included freezing, circling, fleeing, thumping, and vocalizing. Affiliative and comfort behaviors included cuddling, nuzzling, sniffing, allogrooming, and yawning. Nonagonistic behaviors included exploring, self-grooming, eating, and drinking. Inactivity ranged from sitting to lying in a relaxed or stretched position. An ethogram was not performed.

**Contracted toxicology studies.** Rabbits were acquired for planned enrollment in a series of studies including USP pyrogen tests, primary skin irritation tests, and intracutaneous reactivity tests, with 2-wk washout periods and beginning after acclimation to individual polycarbonate rabbit restrainers (Tecniplast, Buggugiate, Italy). The final study, a terminal muscle–subcutaneous implantation test, was to be scheduled when rabbits outgrew the mechanical restrainer (typically approximately 12 wk after arrival).

**Statistical methods.** Weight data were compared by using unpaired *t* tests, with all data passing normality tests (Kolmogorov

and Smirnov) from populations after Gaussian distributions. Behavioral observations were compared between groups by using the Mann–Whitney test. All statistical testing was performed by using Instat 3 for Windows (GraphPad Software, La Jolla, California). Statistical significance was established at $P$ values less than 0.05.

## Results

**Behaviors.** Over the first 2 wk of housing, pen-housed rabbits demonstrated affiliative (body-to-body and nose-to-nose contact, cuddling, allogrooming) and exploratory behaviors, including climbing, rearing and hopping over and under the platform, sharing plastic huts and resting inside PVC tubes. In addition, chasing was seen occasionally during the first 2 wk of pen housing. Over weeks 3 and 4, only affiliative behaviors were noted in the pen-housed rabbits. By the fourth week, the rabbits achieved the minimal body weight for restrainer acclimation training. During week 5, the rabbits were enrolled in the first pyrogenicity tests. A brief episode of chasing was observed during week 5 when rabbits were reintroduced after 48 h of individual separation due to malfunctioning room mechanical ventilation equipment. However, group resting and cuddling behavior returned shortly after the self-limiting chasing behavior. Chasing was noted a total of 10 times, and a single attack was observed, with no evidence of blood on the rabbits or pen surfaces. However, tufts of fur were found occasionally at first observations in the mornings. Stereotypic, bar-chewing behavior was observed in one rabbit during week 5. The following week, a single rabbit was observed to chase, and all rabbits chose to rest far from each other with no obvious evidence of wounding.

All 6 rabbits paired in cages demonstrated affiliative behaviors (body-to-body cuddling, interacting with enrichment, resting) during daily observations, with occasional chasing and 2 observations of attacking with no evidence of aggression including fur tufts or blood throughout 6 wk of housing. Stereotypic bar-chewing was observed once in each group.

The total numbers of affiliative, agonistic, exploratory, and stereotypic behaviors observed did not differ between pen-housed and cage-paired rabbits (Table 1). Chasing and rearing behaviors were observed 2 or 3 times more often, respectively, in rabbits housed in pens compared with pairs in cages (Table 1).

**Wounding.** During week 6 of social housing, all rabbits in both groups presented with approximately 20, dry, granulating and contracting, incision-like, 1- to 2.5-cm linear bite or scratch epidermal and dermal back wounds that penetrated into subcutaneous tissue (Figure 3). These lesions were not apparent on visual exam and were first identified with similar severity in both groups after clipping of fur in preparation for intracutaneous reactivity testing. The injuries might have occurred as long as 28 d prior to identification, in light of wound-healing stages as described in another study involving 12-wk-old, female New Zealand white rabbits.[26]

**Food intake.** Remnants of grain and hay were present in feeding receptacles for both groups when fresh diet was provided daily. Some hay and grain was scattered throughout the pen floor or cage bottom, with a small amount of grain pellets falling through the slatted cage bottom into the litter pan below.

**Body weight.** On arrival, both groups of case-study rabbits were similar in body weight ($P = 0.8405$), with means of 2.34 and 2.36 kg. Rabbits assigned to pen-housing ($P = 0.83$) and cage-pairing ($P = 0.95$) had similar body weights on arrival as that of single-housed, institutional, age- and sex-matched reference rabbits (mean, 2.35 kg). After 6 wk, the paired rabbits (3.31 ± 0.16 kg) weighed significantly ($P = 0.0004$) more than the pen-housed rabbits (2.84 ± 0.16 kg). In addition, pen-housed rabbits weighed significantly ($P < 0.0001$) less than the reference rabbits (3.36 ± 0.24 kg) at this time point, whereas cage-paired rabbits were similar in weight to the reference animals ($P = 0.60$). Percentage weight gain over 6 wk was less ($P < 0.0001$) in pen-housed rabbits (20%) than in pair-housed rabbits (41%), which showed a similar percentage weight gain to that of the reference animals (43%).

**Table 1.** Behaviors observed (no. of incidents) in pen- and pair-housed rabbits during the first 5 wk (35 d)

| | Penned | Paired |
|---|---|---|
| Affliative behavior | | |
| Body-to-body | 28 | 31 |
| Nose-to-nose | 18 | 21 |
| Cuddle | 26 | 28 |
| Allogroom | 12 | 9 |
| Total ($P = 0.61$) | 84 | 89 |
| Agonistic behavior | | |
| Chase | 10 | 4 |
| Flee | 2 | 1 |
| Attack | 1 | 2 |
| Total ($P = 0.64$) | 13 | 7 |
| Explorative behavior | | |
| Rear | 16 | 5 |
| Sniff | 26 | 20 |
| Lick | 15 | 15 |
| Total ($P = 0.11$) | 57 | 40 |

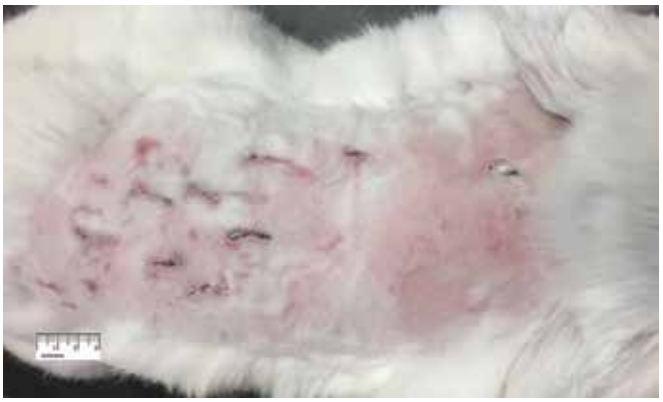

**Figure 3.** Skin wounding lesions

**Contracted toxicology studies.** Both groups of rabbits underwent acclimation training side by side in mechanical restrainers for 3 consecutive days for 30 min daily on the first 2 d and 60 min on the third day. The pen-housed rabbits participated in a single pyrogen test side by side in restrainers for 5.5 h before being separated into single-housing when skin wounds were identified. The rabbits paired in cages never participated in restrainer acclimation or in any tests before being separated once skin wounds were identified and disqualified them for a dermal study.

## Discussion

When housed under natural and seminatural conditions, European rabbits, the wild counterpart of domestic New Zealand white rabbits, demonstrate a repertoire of affiliative and agonistic behaviors that can be anticipated and monitored in laboratory and meat production settings.[10,13,20,21,23,25,30,31,33] The natural,

agonistic, injurious behaviors that led to skin wounds in all of the socially housed rabbits we present here were not noted during the daily observation sessions. The surprising findings of significant skin lesions, which were detected only after clipping fur from the rabbits' backs, disqualified all of these animals from enrollment in dermal toxicology studies. The potential welfare benefits of pair- or group-housing of New Zealand white rabbits must be assessed and weighed against the potential harm associated with injurious aggression or single-housing.[5,8,21]

Establishing social dominance hierarchies among rabbits in the wild and under meat production farming or laboratory conditions predictably involves age-specific, agonistic behaviors established and maintained with physical contact that produces relatively minor wounding from scratches, bites, and fur plucking to more serious injuries such as fractures, castration, and evisceration.[2,10,17,18,20,21] According to one study, aggression decreases as social dominance is established in wild does.[25] Low-ranking captive rabbits confined to cages or pens lack the opportunities to retreat to distant margins of territory that are readily available in the wild.[10,20,25] The complex and gregarious relationships observed between and within both sexes of wild rabbits after hierarchies are established, challenged, and maintained may not be replicable in confined, research, and commercial production facilities.[31]

International animal welfare guidelines encourage social housing of compatible animals, allowing expression of species-typical behaviors while promoting psychologic wellbeing and escape from aggression.[7,12] Not surprisingly, very young rabbits (3 to 4 wk old) in mixed-sex groups prefer to huddle, regardless of the floor space available.[23] Group-housing of young, prereproductive, 11-wk-old does, as we describe in this case study, has been encouraged, with recognition of the risk for aggressive behavior increases with age and onset of sexual maturity.[23,31,32] Adult sexual behavior and maturity, including the first wave of folliculogenesis, occur in female New Zealand white rabbits as early as 12 wk (84 to 90 d) of age.[11,13,19,29] Sexual maturation contributes to aggressive wounding during establishment of dominance hierarchies, especially by the first female that experiences estrous in the group.[16] The numerous skin wounds at various stages of healing in both of our case study groups were first identified at 16 wk of age, thus supporting published findings of potential injurious aggression between female rabbits as early as 12 wk of age.

Most of the behavioral research on group-housed rabbits in captivity has been performed by the meat industry in Europe, with the goal of evaluating behavioral benefits of group housing compared with loss in marketable product and welfare concerns due to wounding as well as social isolation.[31] The age of fattened, group-housed rabbits at slaughter ranges from 35 to 85 d, depending on desired market weight and meat quality. Regardless of housing density, both aggression and wounding in pen-housed, meat-production rabbits increased with age and sexual maturity.[4,10,15,23,24] Regardless of density or enrichment, the severity of wounding increased with age in either sex of rabbit until 80 d (11.4 wk), resulting in a maximum of 21% mortality or culling. In other studies, farmed rabbits butchered at 70 d of age (10 wk) demonstrated no evidence of fighting even at densities as high as one rabbit (mixed sexes) per 0.085 $m^2$ or 12 rabbits per square meter.[32,33] However, extending the slaughter age to 80 d at a similar housing density (1 rabbit per 0.06 to 0.07 $m^2$ or 15 to 16 rabbits per square meter) resulted in 32% wounding (23% minor, 4% intermediate, 5% severe) compared with no wounding when slaughtered at 72 d of age, regardless of whether housed at 6 rabbits in a cage, 10 rabbits in a pen, or 60 rabbits in a large pen.[24,31] Although no injurious aggression and resultant meat damage has been documented before slaughter at 70 d of age at any accepted farming density, the risk of poor meat quality and animal-welfare–compromising wounding convincingly increases after 70 d of age.[13,31,33] Even though our rabbits were housed at much lower densities than the meat industry practices (Figure 2), all of them experienced skin-wounding lesions over their backs at some time between 77 and 105 d (11 to 15 wk) of age. Previous and our current findings thus support 10 wk (70 d) of age as being a potential threshold to ensure lack of skin wounding in socially housed New Zealand white rabbits of either sex, even at extremely low or high housing densities.[24,31-33]

No back-biting or raking behaviors were observed in any of our paired or pen-housed rabbits to account for the skin injuries experienced equally by all animals between 11 and 15 wk of age. This finding might reflect the fact that rabbits primarily express resting and affiliative behaviors during their relatively inactive diurnal phase, in contrast to significantly increased levels of crepuscular and nocturnal activity including agonistic interactions.[1,8,10,16,23,28,31] In a similar study, 11 groups each containing four 14-wk-old (2.8 kg), nonlittermate, female New Zealand white rabbits housed in 1-$m^2$ pens (1 rabbit per 0.25 $m^2$) demonstrated highest aggression at dusk and dawn when establishing a dominance hierarchy during the first 4 d; aggression significantly waned but was not eliminated over the subsequent weeks.[1] In another investigation,[25] 99% of all groups of socially housed does (1 rabbit per 0.1 $m^2$) spent 70% of their time, on average, resting during the day, with aggressive behavior observed less than 1% of the time. Despite such a low incidence of aggression observed during the day, the authors reported that a surprising 52% of the rabbits experienced superficial to severe skin wounds and attributed this unexpected prevalence of wounding to the lack of nocturnal data collection.[25] Juvenile, 5-wk-old, pen-reared Pannon White rabbits housed at a meat-production density of 15 rabbits per square meter (1 rabbit per 0.07 $m^2$) demonstrated aggressive behaviors (biting, chasing, and fighting) only during the night (2300 to 0500). This agonistic behavior increased more than 8-fold throughout the study, as rabbits aged to 10.5 wk.[23] Wild does in nature and domestic does in laboratory housing demonstrate the same behavior, resting peacefully together next to each other during much of the day but choosing to be far from each other and often interacting aggressively during the crepuscular and night hours, thus maintaining the dominance hierarchy and pushing low-ranking does to the peripheral margins of the home range.[10,23] Once a hierarchy is established in a group, only 7% of the dominant does' behaviors in free-living conditions are offensive agonistic actions, but injurious aggression and dominance behaviors persist. By limiting our observations to the work day, we missed the opportunity to identify crepuscular and nocturnal injurious aggressive behaviors that are well documented in rabbits.[17]

As shown in many of the studies cited earlier, social housing promotes interactive, desirable, affiliative as well as unfavorable, but natural, agonistic behaviors. A 5-mo investigation of paired, female New Zealand white rabbits (approximately 9 wk of age at study start) in similarly sized double-wide cages as we used in our case study, showed a reduction in stereopathies (that is, bar biting, pressing or biting of the automatic watering system, head swaying, nose rubbing, floor chewing) compared with singly caged rabbits.[5] Single-housed New Zealand white bucks and does demonstrate a broad repertoire of stereotypic behaviors, including licking, chin marking, overgrooming, bar chewing, head swaying, pawing, restlessness, and boredom

behaviors, which are reduced by social housing and enrichment.[5,8,9] We observed bar chewing only once in each of our groups.

Neither of the 2 housing arrangements we used presented evidence of competition for access to food (grain or hay) or water. Penned and paired rabbits actively manipulated hay from feeders and throughout their floor space. Another study found that juvenile rabbits group-housed in pens and cages at a density of 15 rabbits per square meter from weaning through 10 wk of age gained less weight (presumably due to increased activity) than single-housed rabbits.[15] Our significant findings of a 50% reduction in mean percentage weight gain in pen-housed compared with paired rabbits and in our single-housed rabbits over 6 wk (from 11 through 16 wk of age) support that hypothesis. However, the shorter dark cycle for the pen-housed rabbits compared with caged pairs might have decreased feeding time, even though rabbits in both groups were observed to eat and drink during the day. The reduced weight gain in the pen-housed rabbits delayed their enrollment in pyrogen studies by approximately 2 wk, in light of a minimal body weight requirement of 2.5 kg to begin restrainer acclimation training. The paired rabbits met eligibility criteria for restrainer training on schedule. The correlation of our pen-housed rabbits with significantly less weight gain than rabbits paired in cages should be considered especially when designing developmental and toxicology studies where weight differences might impact study timelines or confound results.[15,23]

Affiliative behaviors and positive welfare indicators such as nuzzling, resting side by side, allogrooming, and eating and drinking together were observed to occur equally in all paired and pen-housed rabbits throughout our case study.[21,28] Pen housing appeared to offer rabbits more space and increased opportunity to demonstrate natural behaviors, such as rearing and chasing, compared with pair-housing in cages. Offensive and defensive agonistic behaviors, such as chasing, fleeing and attacking, overall occurred equally in both groups of our case study.[28] A survey of 1,986 breeding does group-housed in a Swiss meat-production farming program evaluated aggression from the time of weaning through 2 d prior to kindling.[2] The survey identified that 33% of the rabbits experienced wounding that did not compromise breeding efficiency. The authors concluded that the benefits of group housing that promoted natural, social, and enriching behaviors of does outweighed the harm associated with injurious behaviors.[2] After dominance hierarchy was established in pen-housed, female rabbits, the aggressive encounters reduced from 8% to 2%, which can be interpreted as a welfare benefit considering the preponderance of otherwise affiliative or noninjurious behaviors.[10] The benefits of socially housing domestic, 2- to 3-y old female or castrated male rabbits in pens (0.22 to 0.41 $m^2$ per rabbit) compared with single housing in cages included increased comfort and affiliative behaviors (grooming, stretching, yawning) and exploration, with elimination of stereopathies. Rabbits still demonstrated 2.5% undesirable, aggressive behaviors.[21] The densities for pen-housing (1.39 $m^2$ per rabbit) and paired housing (0.51 $m^2$ per rabbit) for our case study provided much more space per rabbit than did the previously cited study.[21] However, the same types of agonistic behaviors, such as biting and raking, even though comprising a low proportion of the behavioral time budget, presumably resulted in our rabbits' skin injuries. Distress and wounding associated with a low incidence of aggressive encounters might arguably be outweighed by the welfare benefit of a preponderance of nonaggressive or affiliative behaviors. Regardless, the skin damage from relatively minor wounding such as we observed may delay or prevent the use of the injured rabbits for their intended research purpose.

Mutual tolerance occasionally is observed between select adult free-ranging, wild, female rabbits within a group especially between a dominant and other submissive rabbits and in young, grazing rabbits and is described anecdotally by facility managers in chronically housed, female, polyclonal-antibody–producing rabbits but was not noted in our 6-wk case study.[16,18,34,35] Given more time together, rabbits in either our pen-housed or paired groups might eventually have developed a relationship of mutual tolerance with or without continued wounding or potentially stressful, but natural, dominant and submissive relationships. If skin wounding had not disqualified rabbits from our time-sensitive dermal toxicology studies or raise animal welfare concerns, additional monitoring over a much longer time might have informed us regarding the development of mutual tolerance and permitted long-term assessment of the harm and benefit of promoting a spectrum of natural affiliative and agonistic behaviors in socially housed rabbits.

Future attempts to socially house female rabbits as pairs in cages or groups in pens might include addition of softwood (for example willow, linden) chew sticks, which reportedly minimize, but not eliminate, aggression and injuries.[15,23,31] Incorporation of infrared video monitoring during the more active, night cycle may allow detection of injurious aggressive behaviors, enabling timely intervention to promote welfare and health and prevent exclusion of study animals due to unacceptable research variables related to trauma.[31]

Despite the undesirability (from a social-interaction cost) of single-housing of mature rabbits, decreasing the abnormal behaviors (for example, excessive fur-licking, bar biting, chewing) correlated with single housing might be possible. Stereopathies have been reduced in single housed male rabbits by using straw and hay cubes as enrichment and manipulanda to chew.[14,22] Single-housed female New Zealand white × Lopp rabbits demonstrated better welfare metrics when offered a box on which to lie instead of hide within.[9] Although isolation due to single housing might predictably result in indicators of poor animal welfare for such a gregarious species, a social experience involving visual and protected contact through mesh might be considered to safely advance a wide array of potentially enriching and desirable behaviors, especially for low-ranking rabbits.[5,8,21] Additional strategies, such as enriched, single-animal exercise pens that promote hopping, exploring, and rearing and potentially reduce or prevent stereotypic behaviors that are correlated with single-housing of rabbits need to be developed and scientifically assessed. This need is especially urgent if more exemptions to social housing rabbits may be scientifically justified specific to injurious aggression and wounding. The primary challenge we face with social housing research rabbits is balancing the undesirable welfare, health, and scientific variables due to injurious aggressive wounding with the benefits promoting potentially long-term, sustainable, desirable, affiliative natural behaviors and positive animal welfare states.

## Conclusions

Aggression is an innate component of both wild and domestic rabbits' behavioral repertoires as observed in natural, semi-natural, farming, and laboratory situations. Known contributors to both affiliative and agonistic rabbit behavior, including age, stocking density, husbandry, housing style, enrichment, and sex should be evaluated when considering group-housing for rabbits. Intact female New Zealand white rabbits older than 10 wk

are gregarious but naturally demonstrate wounding behavior establishing, maintaining and challenging linear rank hierarchy. Regulatory bodies, funding and accreditation agencies, IACUC, researchers, and animal care staff should perform a harm:benefit analysis when considering social housing for New Zealand white female rabbits older than 10 wk. Specific consideration should be given to the possible adverse effects of wounding of cohoused rabbits on animal welfare and research relative to the accepted and published welfare and potential research benefits of social housing.

## References


1. **Albonettl ME, Dessi-Fulgherl F, Farabollinl F.** 1990. Intrafemale agonistic interactions in the domestic rabbit (*Oryctolagus*). Aggress Behav **16:**77–86.
2. **Andrist CA, van den Borne BHP, Bigler LM, Buchwalder T, Roth BA.** 2013. Epidemiologic survey in Swiss group-housed breeding rabbits: extent of lesions and potential risk factors. Prev Vet Med **108:**218–224.
3. **Animal Welfare Regulations.** 2008. 9 CFR § 3.129.
4. **Bigler L, Oester H.** 1996. Group housing for male rabbits. Proc 6th World Rabbit Congr 1996 **2:**411–415.
5. **Chu LR, Garner JP, Mench JA.** 2004. A behavioral comparison of New Zealand white rabbits (*Oryctolagus cuniculus*) housed individually or in pairs in conventional laboratory cages. Appl Anim Behav Sci **85:**121–139.
6. **Council of Europe.** 1976. European convention for the protection of animals kept for farming purposes. Strasbourg, France. 10 March 1976. 1–5.
7. **European Commission.** 2010**.** Directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. OJEU **276:**33–79.
8. **Gunn D, Morton DB.** 1995. Inventory of the behaviour of New Zealand white rabbits in laboratory cages. Appl Anim Behav Sci **45:**277–292.
9. **Hansen LT, Berthelsen H.** 2000. The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*). Appl Anim Behav Sci **68:**163–178.
10. **Held SDE, Turner RJ, Wooton RJ.** 1995. Choices of laboratory rabbits for individual or group-housing. Appl Anim Behav Sci **46:**81–91.
11. **Hutt KJ, McLaughlin EA, Holland MK.** 2006. Primordial follicle activation and follicular development in the juvenile rabbit ovary. Cell Tissue Res **326:**809–822.
12. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
13. **Lehmann M.** 1991. Social behaviour in young domestic rabbits under seminatural conditions. Appl Anim Behav Sci **32:**269–292.
14. **Lidfors L.** 1997. Behavioural effects of environmental enrichment for individually caged rabbits. Appl Anim Behav Sci **52:**157–169.
15. **Maertens L, Van Herck A.** 2000. Performance of weaned rabbits raised in pens or in classical cages: first results. World rabbit science **8:**435–440.
16. **Myers K, Poole WE.** 1961. A study of the biology of the wild rabbit, *Oryctolagus cuniculus* (L.), in confined populations. II. The effects of season and population increase on behaviour. CSIRO Wildl Res **6:**1–41.
17. **Mykytowycz R, Rowley I.** 1958. Continuous observations of the activity of the wild rabbit, *Oryctolagus cuniculus (L.),* during 24 h periods. CSIRO Wildl Res **3:**26–31.
18. **Mykytowycz R.** 1960. Social behaviour of an experimental colony of wild rabbits, *Oryctolagus cuniculus (L.)* III. Second breeding season. CSIRO Wildl Res **5:**1–20.
19. **Mykytowycz R.** 1965. Further observations on the territorial function and histology of the submandibular cutaneous (chin) glands in the rabbit, *Oryctolagus cuniculus* (L.). Anim Behav **13:**400–408, 409–412.
20. **Mykytowycz R, Hesterman ER, Dudzinski ML, Edwards CBH.** 1975. An experimental study of aggression in captive European rabbits, *Oryctolagus cuniculus* (L.). Behaviour **52:**104–123.
21. **Podberscek L, Blackshaw JK, Beattie AW.** 1991. The behaviour of group-penned and individually caged laboratory rabbits. Appl Anim Behav Sci **28:**353–363.
22. **Poggiagliolmi S, Crowell-Davis SL, Alworth LC, Harvey SB.** 2011. Environmental enrichment of New Zealand white rabbits living in laboratory cages. J Vet Behav **6:**343–350.
23. **Princz Z, Dalle Zotte A, Radnai I, Bíró-Németh E, Matics Z, Gerencs Z, Szendrő Z.** 2008. Behaviour of growing rabbits under various housing conditions. Appl Anim Behav Sci **111:** 342–356.
24. **Rommers J, Meijerhof R.** 2010. Effect of group size on performance, bone strength, and skin lesions of meat rabbits housed under commercial conditions. World rabbit science **6:**299–302.
25. **Rommers JM, Reuvekamp BJF, Gunnink H, de Jong IC.** 2014. Effect of hiding places, straw, and territory on aggression in group-housed rabbit does. Appl Anim Behav Sci **157:**117–126.
26. **Simhon D, Brosh T, Halpern M, Ravid A, Vasilyev T, Kariv N, Katzir A, Nevo Z.** 2004. Closure of skin incisions in rabbits by laser soldering. I: wound healing pattern. Lasers Surg Med **35:** 1–11.
27. **Sneddon IA.** 1991. Latrine use by the European rabbit (*Oryctolagus cuniculus*). J Mammal **72:**769–775.
28. **Szeto A, Gonzales JA, Spitzer SB, Levine JE, Zaias J, Saab PG, Schneiderman N, McCabe PM.** 2004. Circulating levels of glucocorticoid hormones in WHHL and NZW rabbits: circadian cycle and response to repeated social encounter. Psychoneuroendocrinology **29:**861–866.
29. **Tablado Z, Revilla E, Palomares F.** 2009. Breeding like rabbits: global patterns of variability and determinants of European wild rabbit reproduction. Ecography **32:**310–320.
30. **Trocino A, Xiccato G, Queaque PI, Sartori A.** 2005. Group housing of growing rabbits: effect of stocking density and cage floor on performance, welfare, and meat quality. Proc 8th World Rabbit Congr 2004 1277–1282.
31. **Trocino A, Xiccato G.** 2006. Animal welfare in reared rabbits: a review with emphasis on housing systems. World rabbit science **14:**77–93.
32. **Verga M, Luzi F, Szendro Z**, 2006. Behaviour of growing rabbits, p 91–97. In: Maertens L, Coudert P, editors. Recent advances in rabbit sciences. Melle (Belgium): Institute for Agricultural and Fisheries Research.
33. **Verga M, Luzi F, Carenzi C.** 2007. Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits. Horm Behav **52:**122–129.
34. **Wyatt J, DiVincenti L.** [Internet]. 2013. Social housing of rabbits. [Cited 29 December 2016]. Available at: http://www.aclam.org/content/files/files/forum2013/aclam_forum_2013_wyatt_divincenti.pdf
35. **Wyatt J.** [Internet]. 2014. Rabbits. Presented at the symposium on social housing of laboratory animals: rabbits. Denver, Colorado, 5–6 October 2014. [Cited 29 December 2016] Available at: https://www.nal.usda.gov/awic/symposium-social-housing-laboratory-animals-2014