Video Article

Methods of Pairing and Pair Maintenance of New Zealand White Rabbits (*Oryctolagus Cuniculus)* Via Behavioral Ethogram, Monitoring, and Interventions

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Abstract

New Zealand White (NZW) laboratory rabbits (*Oryctolagus cuniculus)*, as well as their ancestors the European Rabbit, are a social species that exhibit numerous benefits to being housed accordingly. Although these rabbits are innately gregarious, certain behaviors can still arise when kept in captivity, which if left unchecked, can confound research results or lead to wounding, which in extreme cases can be severe. To prevent these issues, there must be a well-structured plan for the monitoring and maintenance of paired laboratory rabbits. The purpose of this protocol is to present effective procedures for establishing newly paired NZW rabbits as well as methods for successful maintenance. Multiple methods have been tested for the creation of newly paired female rabbits from the vendor, but the most efficacious technique emphasizes capitalizing on the stress bonding from transport, urine marking, pairing in a neutral cage with no forced sharing of resources and a system of monitoring and intervention. To determine the best method of housing paired rabbits in a standard caging environment, data were collected to generate a behavioral ethogram. Behaviors were then quantified as positive, neutral or negative and were tracked across the lifespan of the pair to determine which behaviors indicated pair success or failure. With the newfound knowledge of socially housed laboratory NZW rabbit behavior, enrichment intervention was applied to alleviate aggression and prevent wounding, thus resulting in a higher percentage of successful pairs. Through several years of trialing different pairing methods, the development of the ethogram and the resulting enrichment interventions, understanding of the highly complex social constructs that dominate pair housed rabbit behavior has dramatically increased and allowed for the provision of more species-specific care and increased standards of welfare.

Video Link

The video component of this article can be found at https://www.jove.com/video/57267/

Introduction

The regulations governing laboratory animal care provide well-defined recommendations regarding rabbit social housing. Two of the three primary standards used by AAALAC International to evaluate animal care and use programs describe guidelines for species-appropriate husbandry and housing. The first of these guidance resources, the 8th edition of *the Guide for the Care and Use of Laboratory Animals*[1] (Guide) states that social species such as rabbits should only be singly housed as an exception and single housing should not be the housing standard. The second, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[2], states that rabbits are an inherently social species that should be housed in accordant social situations with the exception of qualifying veterinary or research related reasons. The Office of Laboratory Animal Welfare (OLAW) also mandates that single housing is only appropriate when there is scientific justification that has been evaluated and approved of by the Institutional Animal Care and Use Committee (IACUC), the animals are incompatible or the veterinary staff has a health or welfare concern[3].

In addition to meeting regulatory compliance, peer-reviewed literature provides many examples of the physical and psychological benefits of pair housing NZW rabbits. In order to provide the highest possible standards of animal welfare, species-appropriate housing must be provided while meeting the specific goals of the researcher. Socially housed NZW rabbits engage in more active behaviors[4], display amplified fitness and show diminished levels of gastrointestinal stasis due to increased locomotor behaviors[5]. Physical activity is particularly important for laboratory rabbits as they are prone to osteoporosis[4] when housed in a cage environment. In addition to the physical benefits of social housing, there is a myriad of psychological benefits. Pair-housed NZW rabbits display increased species-specific activities[6], receive enrichment benefits from social stimulation[4], show increased rates of self-soothing activities[7] as well as affiliative behaviors such as allogrooming[8] and exhibit an increased ability to manage anxiety from novel stimuli, i.e. stress buffering[5]. Additionally, in comparison to socially isolated NZW rabbits, socially housed NZW rabbits did not differ in immune function[9] or antibody production[10], however, singly housed rabbits did have higher heart rates[11] and increased white blood cell counts[10] when compared to socially housed rabbits.

Just as the benefits of social housing are well documented in the literature, so are the detriments of social isolation. Single housing of a social species can lead to physiological indicators of stress[12]. Singly housed NZW rabbits spend increased amounts of time being inactive and performing abnormal behaviors[4] and stereotypic behaviors indicative of frustration[13,14,15]. Common stereotypes include: chewing on metal such as cage bars or feeder/waterer, excessive auto-grooming, licking of the cage and self-inflicted negative behavior[16]. If they must be housed singly, additional attention should be paid to counter-balance the stress from social isolation. The Guide recommends providing additional housing space, only housing singly for the minimum amount of time possible, allowing for occasional time in a larger housing situation such as a floor pen and providing additional and varied enrichment opportunities including interaction with other rabbits when possible[1]. AAALAC International additionally recommends providing protected access such that animals can have visual, auditory and olfactory contact with other animals[17].

Given the choice, rabbits prefer to be with other rabbits. Preference testing demonstrated that female NZW rabbits worked almost as hard for limited social contact as they did for food[18]. Multiple other studies have shown that paired rabbits, when given the choice, were observed in physical contact with each other [4,8,19,20]. These studies highlight the significant value rabbits place on socialization with conspecifics. Interestingly, these results do not apply to only female rabbits. When provided with a modified cage divider which was half opaque, half clear with small holes drilled into the clear portion to allow for enhanced but protected social access, male NZW rabbits spent more time in the quarter of the cage that provided the most access to a neighboring male than males in cages without the modified dividers. The male NZW rabbits with protected social access were significantly more active, spending more time in activity and rest behaviors and less on maintenance behaviors such as grooming which is associated with boredom and social deprivation. Males with a modified divider were also less fearful of unfamiliar staff as demonstrated by a reduced latency to approach an unfamiliar hand than males with a standard divider; possibly due to the social buffering effects of interaction with a partner[21]. Additionally, the circadian patterns of activity and rest of the male rabbits with modified dividers became synchronized in a crepuscular pattern, similar to wild rabbits, whereas socially isolated rabbits varied significantly from one another in their patterns of activity and rest[21]. Literature suggests that male rabbits are not ideal candidates for social housing due to aggression post sexual maturity[14,22,23,24]. In wild populations, females are more gregarious than males[6] and multiple females often share a living space while males tend to maintain a larger distance between other males which can lead to increased aggression when placing males in the limited space of a laboratory cage[15,25]. Some studies have attempted to socially house males in a larger pen setting with mixed results[14,22,26,27]. One study attempted pairing males in a cage setting which resulted in all three pairs being separated for aggression[14]. The following protocol is the first to the authors' knowledge that allows for housing males with sibling males successfully in a cage setting into adulthood. There is no pairing of unrelated males or adult males due to the possibility of aggression and no male/female pairings due to the research uses of the colony.

The first step towards rabbit social housing is the establishment of new pairs. This article describes the methods that allowed for the greatest success within this institution of establishing new female pairs and the ethogram used for structured monitoring. The protocol utilizes methods such as urine marking[6,28], stress bonding, providing neutral territory and assuring no forced resource sharing. In wild rabbit colonies, the dominant buck urinates on the rabbits of his breeding group (including kits, juveniles and does) thereby scent marking the rabbits that belong in his social group[29]. This idea has been translated to the laboratory setting by applying freshly collected urine from a male rabbit to the forehead of newly paired females. This imbues the females with a shared scent to indicate to them that they are within the same breeding group and they do not need to compete aggressively. An investigation of using urine marking in creating new female rabbit pairs found that unmarked pairs were more aggressive and exhibited less affiliative behaviors than pairs marked with buck urine[6]. Stress bonding has also been shown to be an effective method of pairing in the companion rabbit community by creating a mildly stressful situation that both rabbits are mutually exposed to in order to encourage bonding[30]. This is typically achieved by a car ride or a walk in a carrier. A theory is that the rabbits will carry over the feelings of security that they received from having a partner during this stressful time into their future bonding experiences and they, therefore, will be more likely to form a successful pair[31]. Pairing new arrival rabbits that have gone through the stress of transport from the vendor creates a natural stress bonding experience that can be capitalized on to assist with the pairing. Once pairs are established, an effective monitoring program must be implemented to track pair behavior over time in order to intervene when necessary. This article highlights the behavioral ethogram that was developed to enable a complete understanding of rabbit social behavior and methods for pair maintenance. Pair maintenance is often a point of difficulty in facilities due to a lack of comprehensive literature describing standard rabbit social interactions and what they may indicate. Rabbit pairs are often separated for interactions that appear to be fighting behavior but are actually normal social interactions that can be monitored and assuaged with proper intervention. This article looks to alleviate this by providing a broad list of behaviors to track and what they may be indicative of. Additionally, environmental enrichment intervention is utilized to aid in the prevention of pair failure. Adequate environmental enrichment may decrease fearful behaviors and allow for better coping with stressors[1], reduce abnormal behaviors[24,32], provide opportunities for essential physiological requirements[33] and overall increase welfare[34]. The results of previous investigation at this institution showed that increasing environmental enrichment facilitates greater success in pair housing by decreasing the aggressive behaviors that arise around the age of sexual maturity (12-17 weeks)[35]. This method has a two-fold benefit; the enrichment creates a distraction to prevent fighting, as well as, allowing for positive interactions through species-typical behaviors.

Protocol

This protocol followed the standards of care developed and utilized by the Unit for Laboratory Animal Medicine (ULAM) of the University of Michigan (U of M), which receives approval and oversight of all animal care procedures and methods by the IACUC. The IACUC is in full compliance with the eighth edition of the Guide. All rabbits were housed in cages that were a minimum of .46 m^2 per cage which exceeds Guide minimum requirements.

1. Methods for the Creation of Female Pairs from Newly Arrived Rabbits that were not Pre-paired by the Vendor.

1. Gather all of the following materials prior to pairing:
    1. Collect urine from a male rabbit. Do this by overturning the liner underneath the cage so that the plastic side is facing up and the urine collects on the top of the liner. Syringe off the urine and store in a sterile conical tube.
    2. Prepare a clean, neutral cage for the pairing to take place in. Remove the center divider so that the pair has full access to two side by side cages (also referred to as one "doublewide" cage). Ensure that this cage contains the following items: a minimum of 2 low-value

enrichment items in addition to 2 high value, destructible enrichment items, a minimum of two hide opportunities such as a perch, hut or box, and 2 separate access points for food and water and two separate piles of loose hay.
3. Fill out the Rabbit Social Introduction Log (**Figure 1**).
4. Prepare a clean spray bottle with fresh water.

2. Unpack the first female from the shipping container.
    1. Weigh the rabbit and perform a nail trim. Mark the rabbit on the tips of the ears with a non-toxic, animal safe marker if no individual identification is available (hair coat, ear tag, tattoo, etc.).
    NOTE: Use a color such as blue or purple that will not be mistaken for bodily fluids such as blood or urine.
    2. Using a gauze pad or cotton ball, apply approximately 1 mL of the previously collected buck urine to the rabbit's forehead and place the rabbit into the neutral cage.
    3. Repeat Steps 1.2 - 1.2.2 with the second rabbit with the exception of marking the rabbit for identification.
    NOTE: Only one rabbit needs to be marked for identification purposes, however, both should be marked with urine.
    4. Monitor the pair continuously for a minimum of 1 h and continue to fill out the Rabbit Social Introduction Log as the pair is monitored, recording each behavior observed at each time point.
    5. Intervene with a squirt of water from the spray bottle only when biting is observed, both rabbits are chasing each other (circling) continuously for over 15 s or fighting behavior (e.g. jousting/lunging) is taking place.
        1. Separate temporarily and physically check each rabbit for evidence of wounding if these behaviors are noted.
        NOTE: Do not spray with water if one rabbit is chasing but the other one is fleeing. The intervention should only be when both rabbits are actively engaged in the chasing. Also, do not intervene too quickly. During this stage, it is essential for the rabbits to establish their dominance hierarchy and frequent interruptions and interventions can delay or prevent this necessary process from occurring.
    6. Contact veterinary staff if minor wounding such as a scratch or pulled fur resulting in a small lesion is observed but do not separate the pair.
        1. Continue to monitor for positive affiliative behaviors indicative of pair success. Refer to 3.2 Positive/Affiliative Behaviors.
    7. Separate immediately if specific types of wounding are noted (see 3.4.3.2), for example actively bleeding lesions or any wounding on or near the genitals or the eyes.

3. Leave the room if after 1 h the pair is stable. Complete two additional 10 min checks throughout the rest of the day (**Figure 2**).
NOTE: Signs of a stable pair include clear dominant and submissive behaviors, resting together, eating/drinking and/or allogrooming (See 3.2 Positive/Affiliative Behaviors). Signs of an unstable pair include both rabbits engaging in clear dominance behaviors, resource guarding or aggressive interactions (See 3.4.1 Aggressive Interactions). The majority of aggressive interactions seen during pairing occur within the first hour, however, if a pair is continuing to display aggressive interactions, monitoring time can be increased.
4. Leave the pair together overnight if the pair continues to be stable after both additional checks.
5. Physically check both rabbits the following morning by feeling under the fur to check for wounding and checking the genitals for lesions.
6. If no significant wounding is observed, perform daily 10 min checks on the pair for the remainder of the week. If the pair is maintained for the remainder of the week, consider these as a stable pair. Document this on the Rabbit Social Introduction Log and generate a Social Housed Interaction and Enrichment Log (**Figure 3**).
NOTE: Pairs are only considered a failure if they demonstrate specific types of wounding (see 3.4.3.2) or if they cannot be left together overnight without new minor lesions being found within two work weeks. Female pairs that were separated due to aggression or research related purposes can be successfully re-paired with the same partner (if separated for research purposes) or with a new female (if separated for aggression), but adult male pairs that were separated can never be re-paired due to the risk of severe aggression. Therefore, the maintenance of male pairs is of utmost importance due to the fact that once separated, they will be singly housed for the rest of their time in the animal facility.

2. Methods for the Creation of Other Types of Pairs including Female Sibling Pairs at Weaning, Female Non-sibling Pairs within One Week of Age at Weaning, Female Weanlings with their Mother, Male Siblings at Weaning or Adults that were Pre-paired from the Vendor.

1. Prepare the neutral caging as described in 1.1.2.
2. Complete step 1.2.1 and place the rabbits together.
3. Monitor for 5 min to ensure compatibility and generate a Social Housed Interaction and Enrichment Log.
4. Proceed to step 1.1 if any fighting behaviors are seen and follow the full pairing process.

3. Observe for Positive/Affiliative, Neutral and Negative Behaviors.

1. Monitor pairs daily and record behaviors observed on the Social Housed Interaction and Enrichment Log.
2. Monitor for Positive/Affiliative Behaviors.
    1. Observe the pair for clear dominance/submission behaviors.
    NOTE: These behaviors include (but are not limited to) behaviors in which one rabbit is displaying dominant tendencies such as chasing, mounting, grooming, chin marking or thumping and the other is accepting and displaying submission behaviors such as fleeing, allowing mounting or grooming or maintaining a chin down posture.
    2. Observe the rabbits sharing resources (food, water, hay).
    NOTE: Consider sharing resources as a positive indicator of pair stability when there is no competition or aggression over the resource.
    3. Observe the pair engaging in allo grooming behaviors in which one rabbit is grooming the other.

NOTE: The rabbit being groomed should accept the grooming. Typically the dominant is grooming the submissive but this can shift to the submissive grooming the dominant which does not indicate a pair breakdown.

4. Observe self-grooming behavior when a rabbit feels secure enough in the shared environment to groom itself.
   NOTE: Consider self-grooming as a positive indication that the pair is stable. If the rabbit felt threatened, it would not engage in this maintenance behavior which requires a vertical sitting posture increasing the rabbit's vulnerability to attack from a hostile partner.
5. Observe the sharing space behavior when the rabbit chooses to be in the same location as their conspecific.
   NOTE: The standard cage setup provides ample space for the rabbits to stay within their own territory if they choose. When they prefer to share space with their conspecific, this is a positive indicator of a stable pair.
6. Observe a rabbit engaging in solo interaction with enrichment items.
   NOTE: The observation that either rabbit is feeling secure enough to engage in interaction with environmental enrichment is a positive indicator.
7. Observe the pair interacting with environmental enrichment items together.
   NOTE: Ensure that both rabbits are mutually engaging in interaction with the environmental enrichment in a non-aggressive and non-competitive manner.

3. Neutral Behaviors.
   1. Observe instances in which no interaction is observed.
      NOTE: There may be a pair that is always observed in a state of neutral or non-interaction. This may not indicate any issues, however, if the pair is consistently neutral and no other positive or negative behaviors are ever observed this pair must be monitored closely. Consistently neutral interactions may indicate that the pair has not yet established a dominance hierarchy and is at a greater risk of fighting and wounding behaviors as time progresses.

4. Negative Behaviors.
   1. Aggressive interactions.
      NOTE: Define aggressive interactions as both rabbits engaging in a negative behavior at the same time. For example, if one rabbit is chasing and one is fleeing, this is not a negative interaction but if both rabbits are chasing each other (circling), this is considered an aggressive interaction because both rabbits are actively engaged in this dominance behavior.
      1. Closely watch for biting, wounding, mutual chasing (circling), resource guarding or leaping at each other in a fighting manner (jousting). Treat these as aggressive interactions that must be intervened.
         1. When the above behaviors are displayed, intervene with a squirt of water from the spray bottle to distract the rabbits from the behavior.
            NOTE: Ensure that the water is never a punishment tool, rather a distraction technique. Typically the rabbits will separate to opposite sides of the cage to groom the water off of their fur.
            1. Don thick gloves to prevent injury and manually separate the pair if the water does not distract from the aggression and physical separation is necessary.

   2. Observe behaviors involving difficulty establishing dominance.
      NOTE: These situations are essential for observers to be able to identify because a pair that cannot establish a proper dominance hierarchy is at a high risk for pair failure and can result in wounding if it is not identified in a timely fashion.
      1. To identify these situations, look for pairs in which both rabbits are exhibiting displays of dominance or the submissive rabbit often struggles to get away from dominance displays.
         For example, if the submissive rabbit does not accept mounting and instead flees away or tries to mount the dominant rabbit.
         1. Provide additional monitoring and enrichment intervention to these pairs to try to assist them in their hierarchy establishment.

   3. Wounding.
      1. Contact veterinary staff immediately for any wounding.
      2. Do not immediately separate the pair unless specific types of wounding are observed, for example, actively bleeding lesions or any wounding on or near the genitals or eyes.
      3. Provide increased enrichment to maintained pairs with minor, superficial lesions such as small scratches or scabs, barbering or hair pulls and monitor. Do not separate these pairs.

   4. When any of these behaviors are noted, place the pair on increased monitoring and enrichment intervention.

5. Monitor for Communication Behaviors that Can Precede Fighting Behaviors.
   NOTE: The following behaviors are all natural ways that rabbits use to communicate their dominance hierarchy with each other which if left unchecked can proceed to fight behaviors. Observation of these behaviors is a good indicator that a pair is working through their dominance establishment and would benefit from increased monitoring and enrichment.
   1. Watch for Chasing/Fleeing Behaviors.
      NOTE: This is a very commonly seen behavior in rabbits to establish dominance. This behavior does not warrant intervention with a spray bottle as long as one rabbit is doing the chasing and the other is doing the fleeing.
   2. Watch for Urine Spraying.
      NOTE: This behavior is most frequently seen in paired adult males but can be observed in paired females as well as in singly housed rabbits. Urine may be sprayed on the cage or on the submissive rabbit.
   3. Watch for Mounting Behaviors.
      NOTE: This is a very typical dominance display with the dominant mounting and the submissive accepting.
   4. Watch for Signs of Barbering.

NOTE: Barbering is usually first observed on the nose, in between the ears or on the back of the neck. Monitor to ensure it does not progress to lesions.

5. Watch for Thumping Behaviors.
   NOTE: Thumping is done for multiple communication reasons. One of those is to display dominance in a decisive intimidation display of repeated thumping towards the submissive rabbit. Thumping may also be done as a sign of submission when accompanied by a fleeing behavior.
6. When any of these behaviors are noted, the pair must be placed on increased monitoring and enrichment intervention.

4. Methods for Pair Maintenance with Environmental Enrichment Intervention.

1. Track the ages of all paired rabbits. Place a note behind the cage card that denotes the age of the rabbits in weeks and update it once per week.
   1. Increase monitoring around weeks 10-20 when problems tend to arise[35]. Sexual maturity begins between 12-17 weeks of age.
2. When negative behaviors (Refer to Section 3.4) or communication behaviors (Refer to Section 3.5) are observed, increase the pair's enrichment to three times per week.
   1. Three times per week provide the pair with a novel enrichment item (one per rabbit) and increased monitoring documented on the Social Housed Interaction and Enrichment Log.
3. If negative behaviors or communication behaviors worsen or new behaviors are observed, increase the pair's enrichment to daily.
   1. Each day (including weekends and holidays) provide the pair with a novel enrichment item (one per rabbit) and associated monitoring documented on the Social Housed Interaction and Enrichment Log.
4. Enrichment guidelines.
   1. Before the use of any enrichment item receive clearance from institutional enrichment committee and/or veterinary staff.
   2. Provide a minimum of one enrichment item per rabbit in each pair that is rotated bi-weekly and loose hay daily[32].
   3. Ensure that all paper and cardboard enrichment items are autoclaved prior to use as per institutional policy.
   4. Remove old enrichment items before adding a new one so that each rabbit does not have more than two items at a time.
   5. Rotate the categories of enrichment to maintain novelty (See **Figure 4**).

Representative Results

Over a 12-month period, 172 NZW laboratory rabbit pairs (62% female, 38% male) were maintained following the above protocol. Of these pairs, only 20% of male pairs and 1% of female pairs had to be separated for fighting as shown in **Figure 5**. The pairs that were separated for fighting behaviors were separated at an average of 18.5 weeks old. The majority of total pairs of both sexes (63%) were separated for research purposes that were not related to pairing, with 29% of pairs still together and only 8% of total pairs having to be separated for aggression.

Of these 172 pairs, stable pairings were able to be maintained well into adulthood with the oldest male pair being 75 weeks old and the oldest female pair being 92 weeks old at the time of separation. Both of these pairs were separated for research purposes and had displayed no wounding and ample displays of affiliative behaviors during their time together. Unrelated females paired on arrival accounted for 21% of the female pairs with the remaining female pairs and all males being weaned together from an in-house transgenic breeding colony.

In total, only 14 of the 172 pairs were separated for aggressive behavior. Of these 14, only two pairs required veterinary treatment beyond monitoring (one required analgesia and one suture). Furthermore, of the 172 pairs in the last year, only two rabbits have had wounding that could not be alleviated with standard therapies or had a significant negative impact on their intended research use.

Rabbit Social Introduction Log

PI Name		Dominant		Submissive		Rabbit Source	
Protocol #		ID #		ID #			
Date		DOB		DOB		Breed	
Initials		Marking		Marking			

TIME	AGGRESSIVENESS					DOMINANCE				NORMAL					SUBMISSION					STRESSED			COMMENTS
	CHASE/RUN	BITE	GUARD	FIGHT	VOCALIZE	MOUNT	GROOM	CHIN MARKING	THUMP	EAT/DRINK	SIT/LAY DOWN	EXPLORE ENRICHMENT	EXPLORE MATE	EXPLORE CAGE	MOUNTED	GROOMED	FLEE	THUMP	CHIN DOWN	WITHDRAWN	EARS BACK	FREEZE	
0-2 min																							
2-5 min																							
5-10 min																							
10-15 min																							
15-20 min																							
20-25 min																							
25-30 min																							
30-35 min																							
35-40 min																							
40-45 min																							
45-50 min																							
50-55 min																							
55-60 min																							
60-70 min																							
70-80 min																							
80-90 min																							
90-120 min																							

Additional Monitoring on Other Side

Figure 1: Rabbit Social Introduction Log. This log allows for detailed tracking of behaviors commonly seen during social introductions. The log is modified from the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Ethogram[37]. Please click here to view a larger version of this figure.

Day of Pairing 10 min check Time: Observer:	
Day of Pairing 10 min check Time: Observer:	
2nd Day 10 min check Time: Observer:	
3rd Day 10 min check Time: Observer:	
4th Day 10 min check Time: Observer:	

Figure 2: Rabbit Social Introduction Log Additional Monitoring Log. This log allows for detailed tracking of behaviors observed post-pairing. The day of pairing requires two additional 10 min checks while the subsequent three days only require one additional 10 min check. Please click here to view a larger version of this figure.

Social Housed Interaction and Enrichment Log

Documentation on this form will begin at time of pairing and be used to track rabbit pair stability. Observations will occur 1-5 times a week for 5-10 minutes to ensure compatibility.			
If pair demonstrates behaviors that require intervention, additional enrichment will be provided at determined frequency and increased pair documentation will occur.			
If pair cannot be maintained, reasons will be documented and separation will occur.			
Rabbit 1 ID #		**Genotype(s):**	
Rabbit 2 ID #		**PI:**	
Date Paired:		**History** (Date behavior occurred, noted behavior, frequency of providing enrichment or separation)	
Sex:			
Relation:			
Enrichment Type: Low Value, High Value, Food Treat or Supplemental Enrichment			
Interaction: Positive Interaction (PI), Neutral (0), Negative Interaction (NI)			

Initials	Date	Enrichment Type (If Provided)	Pair Interaction Observed	Enrichment Interaction Observed	Comments

Figure 3: Rabbit Social Housed Interaction and Enrichment Log. This log allows for detailed tracking of behaviors observed and enrichment intervention provided throughout the course of the pair's life. This document provides a detailed history of behaviors observed and the outcome of the intervention provided. Please click here to view a larger version of this figure.

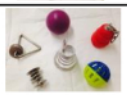
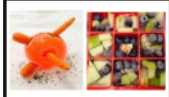
Categories of Rabbit Enrichment		
Low Value - Manipulanda that can be chewed on or moved easily around the cage		
	Balls	Use appropriate size ball for the rabbit. Can be stuffed with other enrichment items. Sanitize according to institutional policy
	Various Toys	Sanitize according to institutional policy
	Wood Blocks and Chew Sticks	Dispose of when soiled, splintered or broken
	Cardboard	Can be placed in the cage or in the cage door for additional enrichment. Dispose of when soiled
High Value - Destructibles. Stuffed with crinkled paper, hay, treats, etc.		
	Carboard Tubes	Dispose of when soiled
	Cardboard Boxes	Dispose of when soiled
	Paper Bags	Dispose of when soiled
High Value - Consumables. Can be fresh, frozen or dried		
	Food Treats	Provide in a way that the rabbit has to work to access the treat to stimulate natural foraging instincts and provide a higher value of enrichment
Supplemental - Provide in addition to other enrichment items, not as the stand alone enrichment		
	Sound Machine	White noise or instrumental music only. Low volume, max 8 hrs/day[36]
	Grooming Brush	Can increase the bond with husbandry staff if the rabbit is receptive to the brushing. Do not force the interaction if the rabbit is uncomfortable.

Figure 4: Categories of Enrichment. This chart describes the different categories of enrichment that are provided to rabbits on a rotating schedule. Please click here to view a larger version of this figure.

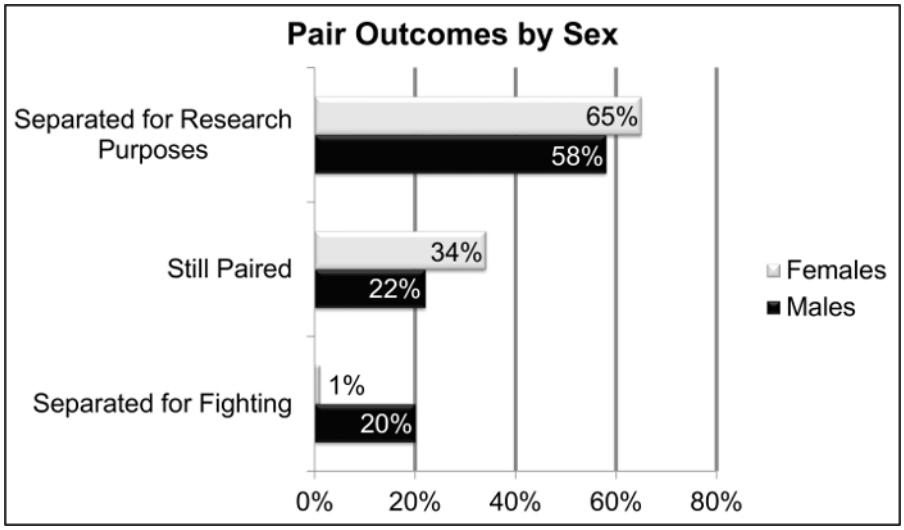


Figure 5: Pair Outcomes by Sex. This figure shows that the majority of males and females were separated for research purposes with the second highest majority of rabbits still being paired. The smallest group of pairs that were separated was as a result of fighting. Please click here to view a larger version of this figure.

Discussion

One of the most difficult challenges facing a rabbit social housing program is the differentiation between species typical aggressive or communication behaviors and problematic fighting behaviors. An in-depth and extensive knowledge of normal rabbit social behavior is required of all husbandry and veterinary staff monitoring the pairing process since many behaviors that appear to be a fight behavior are actually necessary hierarchy establishment behaviors that typically do not result in injury. For example, chasing and fleeing are common, daily behaviors within stable hierarchies that are used to reinforce rank[6,29], but they may be seen as dangerous fight behaviors requiring separation to an untrained eye. Laboratory rabbits spend a significant percentage of their time engaging in aggressive behaviors that contribute to the continued maintenance of the pair hierarchy[38] and wild dominant rabbits require submissive behaviors (e.g. fleeing) from subordinates as frequently as daily[15]. If an observer is unaware of this fact, it could lead to the separation of pairs that are behaving in a species-appropriate manner and do not have a concern that would warrant separation. Due to the intensity of these species-typical behaviors, it can be difficult to determine when to intervene and when to let a pair sort out issues on their own. This is especially important while creating new pairs that are ascertaining their dominance hierarchy for the first time. Too much human intervention can lead to the pair being unable to clearly establish a dominance hierarchy which can lead to pair breakdown. It is vital to have dedicated, educated technicians to monitor the pairs daily to know when behaviors are progressing past the point of normal dominance behaviors. Pairs should always be allowed to express species-typical behaviors when possible[33].

Training is a vital step in the establishment of a successful rabbit social housing program. It is necessary to train and work closely with husbandry staff, veterinary staff and laboratory staff during every step of the pairing process to instruct them on what constitutes a separation worthy behavior and what is acceptable species-typical behavior. Laboratory personnel should be aware of how to contact the veterinary staff to report aggressive behaviors instead of routinely separating rabbit pairs. Proper training will eliminate the unnecessary separation of pairs that could have been kept together with increased enrichment and monitoring.

The failure rate of male pairs was likely higher than that of the females due to difficulty establishing a successful dominance hierarchy within a limited space cage setting. Dominant males require frequent submission activities from the subordinate rabbit[15] and if these are not done the pair might not be able to establish an effective hierarchy, thus resulting in pair failure. Even if the subordinate rabbit is displaying the appropriate submission behaviors, the space constraint of a cage might not allow for adequate fleeing from the dominant[6,39]. This is one of the limitations to pairing males within a cage setting.

Another limitation of this method is that as of the time of publication, alternative scents have not been tested against buck urine for a control. It is possible that a scent that is more readily obtained by facilities that do not have an abundance of male rabbit urine will create the same effect. Previous work done into this concept showed that buck urine was more effective at limiting aggression than doe urine, so it is probable that the odor being from a male rabbit is more significant than it being a novel odor[6], but this has not been tested within this institution. An additional limitation is that the protocol for pairing is only applicable to female new arrivals from a commercial breeder, females weaned with a female sibling or mother, or males weaned with a male sibling at this time. Optimization procedures are currently underway to investigate the most efficacious methods of pairing unrelated females already housed in the vivarium within a standard caging system, but further examination is still necessary before those methods can be confidently recommended. This method also has the restriction of only being applicable to sibling males that have been weaned together and not newly created male pairs. Published reports of creating new paired males in cages have been unsuccessful[14] due to their high levels of aggression towards conspecific males. The maintenance and intervention sections of this protocol however, are applicable to females as well as males (paired at weaning) and as the results showed, can be extremely effective at housing paired males well past sexual maturity and into adulthood. This protocol has also only been tested with NZW rabbits. Additional investigation into other breeds of rabbits is necessary before this protocol can be applied to laboratory rabbit breeds other than NZW. The presented protocol has been tested in Dutch Belted rabbits successfully but not with significant enough numbers that the authors can confidently recommend this method for other breeds. Finally, the recordings were taken at random times during the standard workday. Therefore, behaviors that may be present only

at night were not observed. These behaviors could be beneficial in understanding rabbit behavior since they are a crepuscular species and may display more active behaviors at dawn and dusk[40].

Results show that adhering to the described protocol for social introductions, pair maintenance, and environmental enrichment intervention can lead to a successful NZW rabbit pair housed colony in standard caging with reduced negative interactions that maintain compliance and animal welfare standards. By understanding the behaviors typical to NZW rabbit social interaction and what they indicate, a much more rigorous and effective process was able to be developed which allowed for improvements in the rabbits' quality of life by creating stable pairs and maintaining the pair-housed experience for as long as experimentally possible.

Disclosures

The authors have nothing to disclose.

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