Figure 1. Optimice rack (Animal Care Systems, Inc., Centennial, CO)

A Third-Party Perspective:

Retrospective Analysis of Reproductive Performance in Animal Care Systems Optimice® Ventilated Racks

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Breeding of laboratory mice is an integral part of most institutional animal care programs. Breeding performance plays a crucial role when applying the 3Rs of research, especially the reduction in animal numbers (i.e., fewer breeding animals required). There are many different caging systems and technologies available in the industry, all providing varied microenvironments for the animals. Our institution has been using Animal Care Systems low-flow ventilated carousel Optimice racks (figure 1) to breed transgenic and immune-compromised mice for many years. This study summarizes some of our retrospective data and compares reproductive performance in our colonies to commercial vendor statistics.

Figure 2. Optimice cage

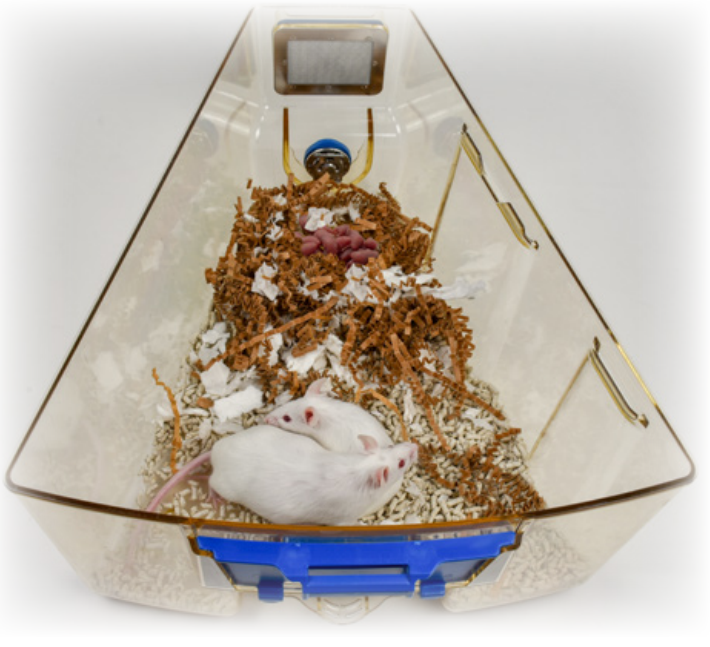

Figure 3. Optimice cage containing mice and pups

Materials and Methods

Animal work in this study was approved by our institutional Animal Care Committee (ACC); mouse breeding was covered under each principal investigator's (PI) ACC-approved protocol. Mice were obtained from several sources and bred in-house as pairs with the exceptions of all the Muc2 mice and a small number of the NOD/ShiLtJ mice, which were bred in trios. Animals were housed in different rooms within the same vivarium. Room temperatures were set at 22°C, and light cycle was set at a 14:10-h light:dark photoperiod. Two sentinels were exposed to dirty bedding from a maximum of 100 cages for 3 months prior to submission. On a quarterly basis, sentinels from each rack were submitted to IDEXX (Columbia, MO) for serology, and to an academic diagnostic lab for parasitology and microbiology. The vivarium is endemic for mouse norovirus (MNV), commensal bacteria and Pasteurella pneumotropica, although not all organisms are prevalent in every room.

Cages contained BioFresh™ Performance Bedding ¼" (BioFresh, Ferndale, WA), Nestlets cotton squares (Ancare, Bellmore, NY), Enviro-dri® (Shepherd Specialty Papers, Watertown, TN) and one red polysulfone shelter (varied sources) (figure 2). Chlorinated reverse osmosis automatic water and rodent chow were provided without restriction. The C57Bl/6 mice for one PI and all Muc2 mice were fed Picolab® Mouse Diet 20 5058 (LabDiet, St. Louis, MO) and the rest of the breeders were provided with Teklad diet 2919 (Envigo, USA). All cages were autoclaved and changed in a cage changing station every 2 weeks. When new litters were noted, cage changing and other disturbances to the cage were postponed for up to 3 days. Pups were weaned at 21 days (Muc2 mice were weaned up to 25 days due to their phenotypically smaller size). Mice were genotyped only after weaning. The floor space of Optimice cages (figure 3) is 75 sq in (484 sq cm). Breeding data were retrieved from a commercial laboratory animal management software program (Mosaic Vivarium, Virtual Chemistry).

Results

Data were collected between July 2015 and December 2018. Reproductive data were summarized for 9 different strains (see table 1), totaling 361 breeding units and 1,331 total litters. Breeders were retired for several reasons: certain PIs retired breeders at a fixed age rather than from attrition, some culled surplus, most retired breeders with >45 days without a litter or if at least two fully cannibalized litters in a row were noted (cannibalized data is included in the data set).

For all strains, average number of pups weaned per litter, as well as wean to born ratio were calculated and compared to published vendor statistics, where available (see table 2). For calculated reproductive indices, all strains performed comparably or were slightly improved in Optimice cages when compared to available vendor statistics. For the NOD/ShiLtJ strain, wean to born ratio was 0.8 compared to 0.9 (vendor); however, the number of pups per litter was 6.4, compared to 4.2 (vendor), an overall increased yield in Optimice cages. The outbred CD1(ICR) strain averaged 9.5 pups per litter in Optimice cages. Although vendor statistics were unavailable for this strain, they are generally considered exceptional breeders (i.e., produce 8 or more pups per litter*).

Discussion

We have productively bred many types of research mice in Optimice racks since 2007, including fragile transgenic and immune- or severely immune-compromised strains. The health status of our mice, including severely immunocompromised strains, has remained unchanged since we transitioned to Optimice cages. In this retrospective study, average number of pups

	Source	Background Strain
NSG™	Jax* 005557	n/a
NOD.Cg-HLAA2	Jax 014570	NOD SCID
HLAA/H2-D	Jax 004191	C57Bl/6J
Rag1	Jax 002216	C57Bl/6J
NOD/ShiLtJ	Jax 001976	n/a
Humanized IAPP	Collaborator/unknown	C57Bl/6J
Muc2	Collaborator/unknown	C57Bl/6NJ
C57Bl/6NCrl C57Bl/6J	CRL† 027 Jax 000664	n/a
CD1(ICR)	CRL 022	n/a

Table 1. Data was collected from 9 different strains from several sources. The first seven listed in the table are immunocompromised strains.
* The Jackson Laboratory, Bar Harbor, ME
† Charles River Laboratories, Wilmington, MA

	No. pups weaned/litter		Wean:Born ratio	
	Optimice	Vendor	Optimice	Vendor
NSG™	6.1	*3-7[1]	0.88	n/a
NOD.Cg-HLAA2	7.5	*3-7[2]	0.98	n/a
HLAA/H2-D	3.9	*Challenging[3]	0.84	n/a
Rag1	5.7	*3-7[4]	0.64	n/a
NOD/ShiLtJ	6.4	4.2[5]	0.80	0.9[5]
Humanized IAPP	7.4	4.9[5]	0.84	0.8[5]
Muc2	4.6	*3-7[6]	0.84	n/a
C57Bl/6NCrl C57Bl/6J	5.0	4.9[5]	0.82	0.8[5]
CD1(ICR)	9.5	n/a	1.00	n/a

Table 2. Comparison of number of pups weaned per litter and wean to born ratio in Optimice cages to vendor statistics, where available.
n/a – data not available
*From the Jackson Laboratory website:
1) Exceptional breeder: Regularly produces 8 or more pups per litter
2) Good breeder: Regularly produces 3-7 pups per litter
3) Challenging breeder: May require special maintenance, exhibit reduced productivity and/or experience some incidence of non-productive matings
**For both the humanized IAPP and Muc2 strains, no vendor statistics were available but since both strains were bred onto a C57Bl/6J and C57Bl/6NJ background respectively, the vendor values for these background strains are included for comparison
[5]Reproductive information for JAX® Ready Strains™

born per litter and wean to born ratio were comparable or slightly improved in Optimice cages for all strains compared to vendor statistics, where there was available data. For the NOD/ShiLtJ strain, wean to born ratio was 0.8 compared to 0.9 (vendor statistic); however, the number of pups born per litter was 6.4, higher than 4.2 (vendor statistic), and an overall increased yield in Optimice cages. The outbred CD1(ICR) strain averaged 9.5 pups per litter in Optimice cages. Comparatively, the Jackson Laboratory website cites exceptional breeders produce 8 or more pups per litter.

Mice in this study were housed in the same type of IVC (Optimice) within the same vivarium; however, since this was a retrospective study of data collected on an on-going basis, cage location in the animal room, room location within the vivarium, diet fed, breeding scheme, and husbandry personnel differences were not controlled. However, no extra animals were acquired, and colony data were collected daily as part of standard vivarium protocol.

Although breeding performance is dependent on many factors that vary between institutions, it is documented that loud or continuous noise, vibration, and bright light[7,8,9,10] can all cause stress and decreased breeding performance in laboratory rodents.[11] The Animal Care Systems racks utilize a unique technology free from motors and blowers; therefore, noise and vibration are eliminated. Light distribution in the carousel rack is less variable compared to library-style racks.[12] The low airflow, which rodents prefer,[13] avoids introduction of air turbulence and high air velocity in the cage. It is probable that the absence of known rodent stressors (noise, vibration, high variation in light exposure and high air velocity and turbulence) and an enhanced cage microenvironment contributes to the consistent breeding performance of our mice when compared to vendor statistics.

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