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Establishment of an Aging Farm of F344/N Rats and C57BL/6 Mice at the National Institute for Longevity Sciences (NILS)

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Abstract

In 1996 the National Institute for Longevity Sciences (NILS) started an aging/aged farm (Aging Farm), an animal farm for producing aging/aged laboratory rodents on inbred strains, F344/N rats and C57BL/6 mice at its Experimental Animal Facility Wing, based on plans prepared by the Laboratory Animal Research Facilities (LARF). The NILS Aging Farm, being well established, began internal supply of aging/aged laboratory rodents in 1999 to promote both aging and longevity science. This report describes development of the NILS Aging Farm under NILS Aging Farm Guide and the effectiveness of the Guide.

Keywords: Aging farm; Aging farm guide; Survivability; F344/N rats; C57BL/6 mice

1. Introduction

NILS was founded to promote aging and longevity science on July 1, 1995. NILS decided to establish an aging/aged farm (Aging Farm), an animal farm for

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producing aging/aged laboratory rodents at the Experimental Animal Facility Wing under the supervision of Director General of NILS and authorization of the Board of Research Directors of NILS. The staff of LARF, a group of facilities for animal experiments at NILS, planned the Aging Farm for NILS.

2. Materials and methods

2.1. Rules for animal experimentation at NILS

LARF prepared a Guideline for NILS to perform animal experimentation with due consideration to animal rights and welfare under the supervision of the Board of Research Directors and the Committee of the Laboratory Animal Experimentation.

2.2. Experimental animal facilities of NILS

Experimental animal facilities of NILS consist of two 720-m^2 floors at one of the building wings. The second floor is enclaved with a barrier system (BS) where specific pathogen free (SPF) laboratory rodents, rats and mice are housed. Formally, on the same floor there were three BS-based experimental rooms, eight BS animal rooms, and one semi-clean stock room. Only one autoclave was placed between the clean areas and semi-clean area. When the A/F was established with the existing BS, all experimental rooms were remodeled into animal rooms equipped with two autoclaves and one EO-gas sterilizer. Sterilized air is supplied to all animal rooms through High Efficiency Particulate Air (HEPA) filters under the positive pressure with 15-mm water. Pressurized air is directed from the clean area to the semi-clean area and supplied in the form of laminar flow to each animal room.

Laboratory rodents are bred in an environment intended to be free from infectious disorders by blocking potential routes of microorganisms such as HVJ and MHV. Standard operative procedures (SOP) are put in place to minimize microorganic contamination in the BS environment. For instance, air shower and airtight partitions/doors, in addition to autoclaves (Sakura Seiki Co., Tokyo) and EO-gas sterilizer (Sakura Seiki), provide effective separation from the ambient.

2.3. NILS Aging Farm Guide

LARF prepared a Guide for maintenance of BS and establishment of Aging Farm (Table 1). When an Aging Farm Guide is appropriately prepared and strictly followed, reproducible results can be obtained in respect to the survival of inbred strains of laboratory rodents. Reproducible information on the survival is important to constantly, stably produce aged laboratory rodents Table 1.

Under the NILS Aging Farm Guide, illumination is provided in a 12-h lighting (08:00–20:00) and 12-h dark cycle. Temperature is controlled at between 22 and 23°C with a notch of 0.5 to 1°. Humidity, however, is not regulated.

Table 1
NILS Aging Farm Guide

Animal Room	11 in total	6 for rats	4 for mice	1 for rats and mice		
Temperature	22–23°C		±0.5–1°C range	Auto monitor		
Humidity	Not regulated		57 ± 5°C	Auto monitor	Seasonal difference	
Illumination	12L12D	08:00–20:00				
Air supply	HEPA filter	Positive pressure with 15 mm H_2O				
Air flow	One way (laminar flow)					
Housing racks	Rats: S-1395RC, S-1395RS	Mice: S-1395MC, S-1395MS				
Animals	Rats: 2/TPX cage	F344/N				
	Mice: 5/TPX cage	C57BL/6		SAMR1TA DDD/Jah	SAMP6/Ta C57BL/6//Jah	SAMP8/Ta
Sterilization	Hausing materials	A/C				
	Diet	Irradiation				
	Water	A/C				
	UV lamp	Passage				
Diet	MR stock	Mouse/rat	Protein 18%	3.1 kcal/g		

Housing racks are measured on the basis of the size of animal rooms to allow the highest level of capacity and easy care. The most effective width of a rack at LARF is 1395 MM. Racks, ordered to Showa Kagaku Co. Ltd., Tokyo, have five shelves for rats and seven shelves for mice. Rats can be contained in five cages on each shelf or 25 cages in total. Mice can be accommodated in seven cages per shelf or 49 cages in total. Each of two types of cages is designed to house two rats and five mice respectively and made by Clea Japan Co., Tokyo. Rotary-type cage washer made by Seiwa Sangyo Co., Tokyo, cleans these cages.

Sterilized air is passed through HEPA filters from the punched board at the backside of each shelf. At the front of the shelf is a bar to prevent a protrusion of cages. The bar (LARF/S system) can keep cages in place on the shelves even when the rack falls down.

Diet is Labo MR Stock, containing 18% protein, prepared by Nihon Nousan Co., Yokohama. The stock is sterilized by irradiation. Water is supplied in a 250-CC bottle by Okazaki Sangyo Co., Souka. Nesting material is made of wooden chip (White Flake), purchased from Charles River Japan Co., Yokohama. Before usage, all housing cages with nesting material and water bottles are put in aluminum cans (Showa Kagaku Co.) for autoclave sterilization. All wears and equipment for caretakers are similarly sterilized through EO-gas sterilizer.

2.4. Aging Farm

Our mission is to establish and maintain an Aging Farm of small aging laboratory rodents as indispensable animals for the progress of aging and longevity sciences. Our A/F project started on June 1, 1996, under the NILS A/F Guide specified by LARF. LARF chose F344/NSlc (F344/N) rats and C57BL/6CrSlc (C57BL/6 or B6) mice as its laboratory strains of aging rodents because of their popularity in aging science. Especially, F344/N rats have many advantages to long-term housing thanks to their long median life span and small body size. B6 mice are characterized by low incidence of mammary tumor.

The first animal group was introduced for microbiological monitoring from SLC Co., Hamamatsu. Then, 4-week-old colonies of these two strains were also purchased from SLC. New colonies of the same age and strains were added every month. The first colonies reached the thirtieth month in January 1999, being followed by successive groups. The first three colonies have been monitored to plot survival curves. Intramural supply of aged animals started with the fourth colonies.

DDD/Jah mice and C57BL/6//Jah mice were procured as breeding nuclei from the National Institute of Animal Health, Tsukuba in July 1996, and SAMR1TA, SAMP6/Ta and SAMP8/Ta from Takeda Pharmacology Co., Osaka in August. These five strains have been reproduced while their survival curves have been kept on record.

Animals are housed with SPF conditions in the BS as per the NILS Aging Farm Guide. F344/N and B6 are monitored for any decrease in the number of constituents of respective cohort. Other animals are checked for the number of living days.

During our observation, both purchased and reproduced animals have developed various interesting disorders in their aging processes. These disorders will be reported in detail on later days.

2.5. Care taking

Maintenance of the Laboratory Animal Facilities Wing is performed by contract staff from Sankyo Labo Service Co., Tokyo. They are Messrs. Kazuo Shiokawa (1996/6/1-), Seiji Tamuta (1996/6/1-), Tetsuya OMAE (1996/6/1-), Katsuhisa Suzuki (1996/6/1-1997/2/28), Suetoshi Takahashi (1996/6/1-1996/12/31), Narumi Hirabayashi (1996/12/1-), Hiromichi KONDO (1997/4/1-), Masaaki Yajima (1998/2/1-). Their devotion to care and maintenance have made it possible to establish the Aging Farm successfully.

3. Results

This report deals with only the survival of F344/N rats and B6 mice since the way survivability (SvA) is calculated is different between these two strains and other five strains. The purpose is to evaluate the efficiency of NILS Aging Farm Guide for three groups of animals that are divided by the date of birth (Group-1: June 25, 1996: Fig. 1, Group-2: July 23, 1996, and Group-3: October 1, 1996).

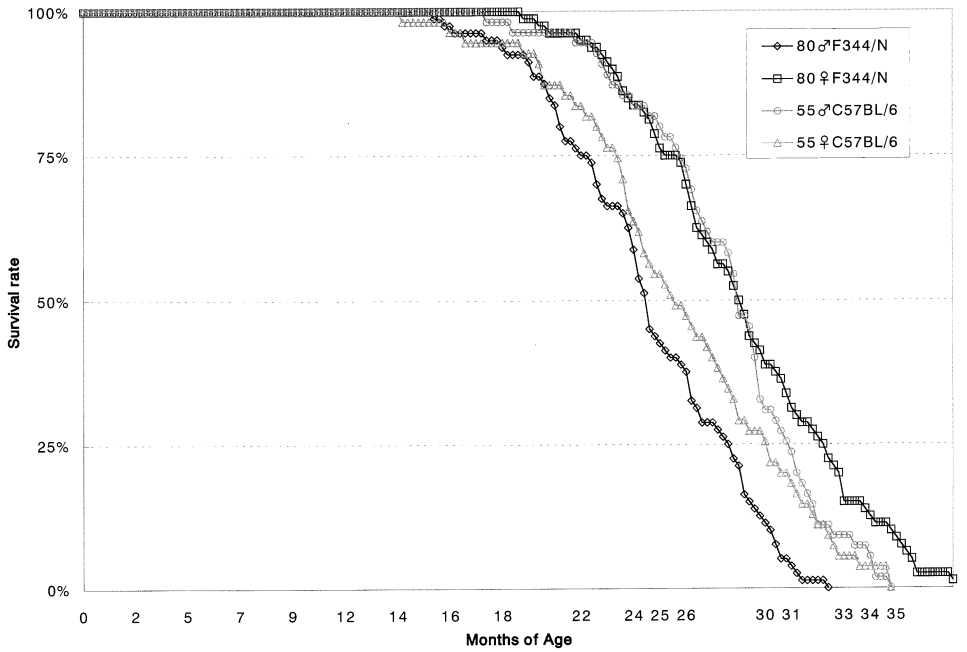

Fig. 1. Survival of F344/N rats and C57BL/6 mice at NILS Aging Farm.

As summarized in Table 2, SvA is compared as of August 31, 1999 for average survival and 75-, 50- and 25%-survival.

3.1. F344/N rats

3.1.1. Average survival

For Group-1 males ($n = 80$), the first death appeared on the 467th day of age, and the last death on the 992nd day of age. For females on the same group ($n = 80$) the first death occurred on 585 days of age, and a few rats are still alive beyond 1155 days of age. The average survival of Group-1 males was 754.6 days, while that of Group-1 females has not been identified.

For Group-2 males ($n = 55$), the first death appeared on the 483rd day of age, and the last death on the 942nd day of age. For females on the same group ($n = 55$) the first death occurred on the 553rd day of age, and some rats are still alive beyond 1112 days of age. The average survival of Group-2 males was 742 days, while that of Group-2 females has not been identified.

For Group-3 males ($n = 109$), the first death appeared on the 462nd day of age, and the last death on the 1024th day of age. For females on the same group ($n = 110$) the first death occurred on 441 days of age, and many rats are still alive beyond 1049 days of age. The average survival of Group-3 males was 765 days, while that of Group-3 females has not been identified.

3.1.2. 75%-Survival

In Group-1, 75%-SvA of male attained on the 668th day of age, and that of female, the 769th day. In Groups 2 and 3, 75%-SvA of male was the 658th and 678th day, and female, the 743rd day and 746th day, respectively.

3.1.3. 50%-Survival

In Group-1, 50%-SvA of male attained on the 749th days of age, and female, the 862nd day. In Groups 2 and 3, 50%-SvA of male was the 738th and 752nd day, and female, the 852nd and 873rd day, respectively.

3.1.4. 25%-Survival

In Group-1, 25%-SvA of male attained on the 860th day of age, and female, the 979th day. In Groups 2 and 3, 25%-SvA of male was the 843rd and 850th day, and female, the 938th and 951st day, respectively.

3.2. C57BL/6 mice

3.2.1. Average survival

For Group-1 males ($n = 55$), the first death appeared on the 541st day of age, and the last death on the 1077th day of age. For females of the same group ($n = 55$) the first death occurred on the 419th day of age and the last on the 1099th day of age. The average survival of Group-1 males was 860.5 days, while that of Group-1 females was 791.3.

Table 2
Survival of F344/N rats and C57BL/6 mice at the NILS Aging Farm[a]

Strain/species (date of birth)	Sex	Number of animals	Range (days)	Average (days)	75% survival		50% survival		25% survival	
F344/N/Rats					Months	Days	Months	Days	Months	Days
(1996/6/25)	Male	80	467–992	754.6	21.97	668	24.63	749	28.28	860
	Female	80	585–1155+	–	25.29	769	28.35	862	32.19	979
(1996/7/23)	Male	55	483–942	742	21.64	658	24.27	738	27.27	843
	Female	55	553–1112+	–	24.43	743	28.02	852	30.85	938
(1998/10/1)	Male	109	462–1024	765	22.30	678	24.73	752	27.95	850
	Female	110	441–1049+	–	24.53	746	28.71	873	31.27	951
C57BL/6/Mice										
(1996/6/025)	Male	55	541–1077	860.5	26.31	800	28.58	869	30.81	937
	Female	55	419–1099	791.3	23.12	703	25.62	779	29.73	904
(1996/7/23)	Male	53	251–1101	855.4	24.83	755	29.37	893	32.00	973
	Female	53	272–1020	799.9	23.45	713	26.93	819	30.06	914
(1996/10/1)	Male	108	468–1056+	–	24.96	759	27.98	851	31.67	963
	Female	102	464–1056+	–	21.57	656	25.65	780	28.54	868

[a] As of August 31, 1999.

For Group-2 males ($n = 53$), the first death appeared on the 251st day of age, and the last on the 1101st day of age. For females of the same group ($n = 53$) the first death occurred on the 272nd day of age, and the last on the 1020th day of age. The average survival of Group-2 males was 855.4 days, while that of females was 799.9.

For Group-3 males ($n = 108$), the first death appeared on the 468th days of age and some mice are still alive beyond 1056 days. For females of the same group ($n = 102$) the first death occurred on the 464th day of age and some mice are still alive beyond 1056 days of age. The average survival of Group-3 has not been identified.

3.2.2. 75%-Survival

In Group-1, 75%-SvA of male attained on the 800th day of age, and female, the 703rd day. In Groups 2 and 3, 75%-SvA of male was the 755th and 713th day, and female, the 759th and 656th day, respectively.

3.2.3. 50%-Survival

In Group-1, 50%-SvA of male attained on the 869th day of age, and female, the 779th day. In Groups 2 and 3, male 50%-SvA was the 893rd and 851st day, and female, the 819th day and 780th day, respectively.

3.2.4. 25%-Survival

In Group-1, 25%-SvA of male attained on the 937th day of age, and female, the 904th day. In Groups 2 and 3, 25%-SvA of male was the 973rd and 963rd day, and female, the 914th and 868th day, respectively.

4. Discussion

The results of SvA, including average survival and 75-, 50- and 25%-survival of F344/N rats and C57BL/6 mice in three age groups are highly reproducible. It is particular so for male rats (Table 2). Unfortunately average survival has not been identified for female rats because of their longer life span than that of male rats (Sprott and Austad, 1995). A decrease in survival curves is gradual, in particular for C57BL/6 mice. With an invasion of infectious diseases and/or conventional (non-SPF) housing conditions (Solleveld, 1987), an abrupt decrease or an early negative slope in a linear fashion could have been detected. In contrast, the curves prove the appropriateness of the NILS Aging Farm Guide (Table 1) to fulfill the NILS mission and the preciseness of care extended by the contract staff in maintaining animals and facilities. SvA of the two strains under the NILS Aging Farm Guide environment can serve as a new, strain-specific, sensitive parameter in terms of their biological characteristics.

It should be noted that the latest results by LARF-NILS on SvA of F344/N appear different from other reports and references on F344 rats (Table 2). Average survival of male, in particular, seems to be shorter than that in other reports and references (Festing, 1979). We are under investigation to identify the nature of the gap with special attention to the differences among sub-lines of F344.

In our results, SvA had clear sex difference for F344/N rats. Females of this strain survived longer than males of the same strain. Average survival of females has not been identified even after 1155 days of age. In addition, male reached 75-, 50- and 25%-survival earlier than females by more than a hundred days. F344/N SvA under the NILS Aging Farm Guide is, thus, concluded to be different between sexes.

Similarly, clear sex difference in SvA was detected for C57BL/6 mice. Of this strain, males survived longer by two months than females. This shows a sex difference but in the opposite combination when compared with F344/N from NILS Aging Farm.

In addition, an interesting similarity in the days of age should be noted between average survival and 50%-SvA of male F344/N rats in all Groups and C57BL/6 mice in Groups 1 and 2. This suggests that 50%-SvA on each strain can be one of the most important and convenient parameters to establish or evaluate an Aging Farm for laboratory strains of rodents.

The contrast in SvA by sex between F344/N and C57BL/6 seems to reflect differences related to either species or strains. For further clarification, SvA should be compared among various strains and species. It will also help to determine common factors among individual strains or species. This process will eventually improve biological significance of these rodents as animal models.

This report is intended to present a reproducible SvA for both F344/N and C57BL/6 and show that the Aging Farm Guide planned by LARF and adopted by NILS is effective for the production of aging/aged laboratory rodents to contribute to longevity sciences.

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