



NOTE

Laboratory Animal Science

Effects of aging and delivery order on the breeding capacity of F344/N rats

Shinsuke H. SAKAMOTO¹⁾, Kazutoshi NISHIJIMA^{2-4)*}, Tamio OHNO⁵⁾ and Shin TANAKA⁶⁾¹⁾Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan²⁾Center for Animal Resources and Collaborative Study, National Institutes of Natural Sciences, Aichi, Japan³⁾Department of Physiological Sciences, National Institutes of Natural Sciences, Aichi, Japan⁴⁾Department of Physiological Sciences, SOKENDAI (The Graduate University for Advanced Studies), Aichi, Japan⁵⁾Division of Experimental Animals, Center for Promotion of Medical Research and Education, Graduate School of Nagoya University, Aichi, Japan⁶⁾Formerly Animal Facility for Aging Research, National Center for Geriatrics and Gerontology (NCGG), Aichi, Japan

ABSTRACT. Reproductive performance affects the efficiency of the production of animals. Therefore, knowing the reproductive properties of each species or strain of animal is important for proper management of breeding stocks. To elucidate the reproductive properties of female F344/N rats, frequently used for longevity and gerontology research, we monitored the breeding duration, number of deliveries, litter size and weaning rate of their pups. The first delivery was observed at 2.9 ± 0.1 (mean \pm standard deviation) months of age (M) and the last delivery was at 15.1 ± 1.8 M. The number of deliveries was 10.4 ± 2.8 (range of 7–16) within the life span of the mother (24.7 ± 5.6 M). The litter sizes at the third (10.0 ± 3.0) or fourth (10.3 ± 2.7) deliveries were higher than for other deliveries. The breeding outcome declined after the fifth delivery yielding reduced litter size or weaning rate, number of delivered mother. These results suggests that though female F344/N rats are able to deliver more than 10 times, the reproductive performance lowered after fifth delivery.

KEYWORDS: aging, breeding capacity, F344/N, inbred rat

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Reproductive efficiency is a major concern in animal breeding, not only in relation to farm animals but also laboratory animals. In addition, an adequate lifetime breeding plan based on a breeding capacity should improve the level of welfare for captive animals. Therefore, it is desirable that we could obtain the information about breeding capacity of all captive animals, e.g. Zoo animals, companion animals, livestock, and laboratory animals. However, as we know, there are few studies reported breeding capacity of laboratory murid rodents with long lifespan. It may contribute to keep not only laboratory strains but also wild rodents especially after successfully constructing a founder population.

With laboratory animals, it is necessary to keep large colonies for stable supplies of animals of various strains, ages and sex. The breeding conditions of these animals are strictly controlled in terms of temperature, humidity, cleanliness, and lighting, and consequently they are generally bred in cramped conditions. As a result, good reproductive performance—determined by pregnancy rate, litter size, weaning rate, reproductive duration and number of deliveries—is critical for breeding efficiency. Since the reproductive properties of animals change with age [4, 6, 7, 9, 16], it is also important to determine the appropriate retirement age of breeding animals.

F344/N is an inbred rat strain frequently used for longevity and gerontology research because of its long life span [12, 13]. This long life span is also useful for examining reproductive status and properties throughout the animal's life. Aging changes in the rat have been analyzed from various perspectives [7, 8, 14]. We have previously shown the aging-induced changes in the number and population of follicles and corpora lutea in the ovary of female F344/N rats and observed follicles and corpora lutea in the rat ovary at 36 months of age (M) [8]. However, as Sone *et al.* have reported [11], the cornified cell (estrus) phase, identified by vaginal smear cytology, ceased around 16.4 M. These results indicate that there is a temporal gap between the morphological and physiological changes in the reproductive properties with aging; and furthermore, these changes might differ from breeding capabilities.

*Correspondence to: Nishijima, K.: kanish@nips.ac.jp, Center for Animal Resources and Collaborative Study, National Institutes of Natural Sciences, 38 Nishigonaka Myodaiji, Okazaki, Aichi 444-8585, Japan

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The current study was conducted to elucidate effects of aging and delivery order on the breeding capacity of F344/N rats by monitoring the breeding duration, number of deliveries, litter size, and weaning rate of their pups.

Animal experiments were performed in accordance with the guidelines for animal research of Japan SLC Inc.

The F344/N rats were kept in the animal rooms of the strain colony maintaining facilities at Japan SLC Inc. (Hamamatsu, Japan). The barrier system in the animal rooms was supplied with positive airflow sterilized with HEPA filters. The rooms were regulated in illumination (08:00–20:00, light), temperature (23–25°C) and humidity (45–65%). The rats were fed with MR-A1 food pellets (Nihon Nousan Kogyo, Yokohama, Japan) sterilized by autoclaving, and given acidified water (pH 2.5–3.0) *ad libitum*. The animals were housed in metal wire-bottomed cages.

The females were mated with sibling lifelong partner males, which is standard strategy to maintain the inbred strain. The females were kept with the males for free mating except nursing periods. Pregnant females appraised by palpation were moved to aluminum-bottomed cages with sterilized wooden chips for nursing until pup weaning began (3 weeks after delivery).

Eleven female F344/N SLC rats were selected based on their reproductive performance in the first 2–3 deliveries. The age at first and last deliveries, number of deliveries, litter size, and weaning rate of the pups were monitored throughout the life span of the 11 mother rats.

Cases in which there were traces of delivery from pregnancy-confirmed mothers but no pups were found were categorized as cannibalism. Non-nursing indicates cases with confirmation of pups at delivery and occurrence of cannibalism and/or neglect thereafter, and thus data about litter size and weaning rate was available.

One-way ANOVA followed by Bonferroni test was employed to reveal any significant differences in litter size and number of weaning offspring between an ordinal number of deliveries. Regression analyses were made between all pair combinations of the following measures: life span, age at first or last delivery, breeding duration, number of deliveries, delivered pups, litter size and weaning rate.

The longest life span was 30.9 M, while the shortest was 16.4 M. The average life span of the 11 rats was 24.7 ± 5.6 (mean \pm standard deviation) M (Table 1). No significant correlations were found between life span and last delivery ($R^2=0.3205$), breeding duration ($R^2=0.2814$), number of deliveries ($R^2=0.0446$).

The youngest delivery was observed at 2.7 M (fertilized at 2.0 M), while the oldest was at 17.5 M. The average age of the mother at the first delivery was 2.9 ± 0.1 M, and at the last delivery it was 15.1 ± 1.8 M (Table 1).

The number of deliveries throughout the life span of the rats ranged from 7 to 16, and the average was 10.4 ± 2.8 (Table 1).

The reproductive duration was calculated by subtracting the age at first pregnancy from the age at the last delivery. The breeding durations ranged from 9.1 to 15.2 M and the average was 12.8 ± 1.9 M (Table 1). The ratio of breeding duration to life span was $53.6 \pm 10.5\%$.

The litter size was highest in the third (10.0 ± 3.0) and fourth (10.3 ± 2.7) deliveries, and showed a downward tendency after the fifth delivery (Fig. 1). The number of delivered mothers decreased gradually after 8th delivery (Table 2). The occurrence of non-nursing dragged down the weaning rate (e.g. 2nd and 5th delivery, Table 2), which was over 90% without the non-nursing deliveries (e.g. 1st and 3rd delivery, Table 2).

Because of the decrease of delivered mothers and litter size, the total breeding outcome followed a course of decline after fifth delivery (Fig. 1).

The present study reports effects of aging and delivery order on the breeding capacity of female F344/N rats in terms of number of deliveries, litter size, and weaning rate of their pups. The first delivery at 2.9 M (fertilized at 2.2 M) lagged behind the development of estrus cycles at 1 M, as reported by Sone *et al.* [11]. This might indicate that female F344/N rats reach puberty at 1 M and attain sexual maturity at 2 M. The last delivery corresponded to the cessation of the estrus cycles at around 17 M [11]. Past this age, the number of corpora lutea decrease and the atretic follicles increase in the rat ovary [8]. Furthermore, the survival rate of

Table 1. Breeding performance of each female F344/N rat over the life span

Individuals	Life span	Age at first delivery (fertility)	Age at last delivery	Breeding duration ¹⁾	No. of deliveries	Average litter size	Weaning rate (%) ²⁾	Cannibalism / Non-nursing
1	30.5	2.9 (2.2)	15.9	13.7	9	9.7	98.5	2 / 0
2	30.9	3.0 (2.3)	15.6	13.3	9	7.4	97.0	0 / 0
3	19.5	2.9 (2.2)	15.5	13.3	13	7.8	89.5	2 / 1
4	27.3	2.8 (2.1)	15.2	13.1	13	6.9	89.5	2 / 2
5	22.8	3.1 (2.4)	14.2	11.9	12	6.8	96.0	1 / 1
6	21.5	2.7 (2.0)	16.9	14.9	16	7.0	95.9	2 / 1
7	30.2	3.0 (2.3)	17.5	15.2	8	5.3	93.8	2 / 1
8	25.9	3.0 (2.3)	16.7	14.4	10	6.6	93.9	0 / 0
9	16.4	2.8 (2.1)	13.0	10.9	7	6.6	91.3	0 / 0
10	30.4	3.1 (2.4)	13.9	11.5	8	8.1	75.4	1 / 1
11	16.9	2.9 (2.2)	11.2	9.1	9	6.2	98.2	0 / 1
Mean	24.7 ± 5.6	2.9 ± 0.1 (2.2 ± 0.1)	15.1 ± 1.8	12.8 ± 1.9	10.4 ± 2.8	7.1 ± 1.1	92.6 ± 6.5	

¹⁾Duration between the age at first pregnancy and the last delivery. ²⁾Delivery with cannibalism was excluded. Age and duration is shown in months (M).

female F344/N rats begins to decline around this age [12, 13], which may be possibly related to diseases associated with depression of estrogen levels [2, 15]. In this study, two out of the eleven rats died at 16.4 and 16.9 M. The average life span of the rats was 24.7 ± 5.6 M, which was shorter than previously reported for nulliparous females [12, 13]. This difference indicates that bearing and nursing pups exerts a burden on the mother's body [5], even though no significant correlation was found between life span and breeding outcomes in the present study. Though a significant linear relationship between median age of death and effective end of reproduction was revealed in 22 mouse strains [1], we could not find high correlation between life span and age at the last delivery in individual rats in the present study.

The most prolific mother in the present study gave birth to 98 offspring within 15 deliveries (excluding a delivery with cannibalism) and brought up 94 offspring. The least prolific rat gave birth to 32 offspring within 6 deliveries (excluding two deliveries with cannibalism) and brought up 30 offspring. The female F344/N rats held their breeding capabilities until 15.1 M and delivered 10.4 times in their lives, on average. However, the litter size after the seventh delivery was lower (<5.6) than that of former 6 deliveries (>7.2) and the total number of weaned offspring obtained from the 11 mothers after the eighth delivery fell to less than one third of the peak, that of the fourth delivery (108 pups). These results suggest that using female F344/N rats that have delivered more than six times would be inefficient for breeding. Additionally, as instances of cannibalism were observed after fifth delivery, we suggest that female F344/N rats should be retired after the fourth nursing to achieve more efficient breeding. The aging properties are known to be different among species or strains [3, 10], and therefore elucidation of the reproductive properties in each strain of animal is needed. In this study, the females were mated with sibling lifelong partner males, which is standard strategy to maintain the inbred strain. Therefore, variations in male characteristics and/or female-male relationships could also modify breeding outcomes of females. In the next step, experiments taking into account the effects of male characteristics on breeding outcomes of females are desired.

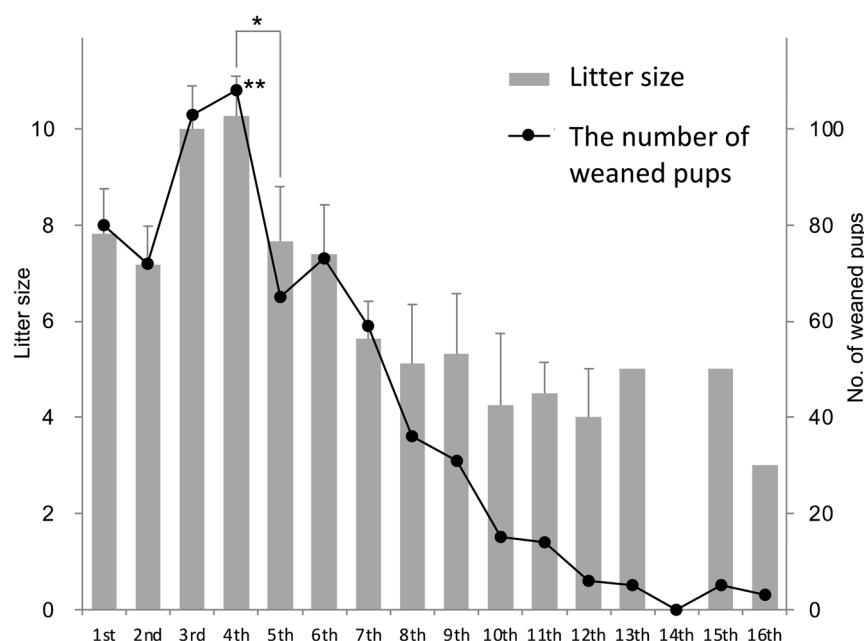


Fig. 1. Breeding outcome from 11 female F344/N rats. Litter size ($*P<0.05$) and the number of weaned pups ($**P<0.01$) in the 4th delivery were significantly higher than those in the 5th delivery, respectively. N/A: Not available because of occurrence of cannibalism.

Table 2. Breeding performance and age at each time of delivery

No. of delivery	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
Delivered mother (out of 11)	11	11	11	11	11	11	11	10	8	5	4	4	3
Cannibalism / Non-nursing	0/0	0/1	0/0	0/0	2/1	1/0	0/1	1/3	2/0	1/1	0/1	2/0	2/0
Weaning rate (%)	94.8	85.5	94.7	95.0	82.0	99.2	90.0	62.9	96.7	71.9	75.0	73.3	100
Age at delivery (months of age)	2.9 ± 0.1	4.3 ± 0.4	5.2 ± 0.4	6.3 ± 0.6	7.5 ± 0.7	8.8 ± 0.8	10.4 ± 1.4	11.4 ± 1.7	12.5 ± 2.3	12.1 ± 0.6	12.9 ± 0.7	14.0 ± 0.5	15.2 ± 0.0
Interval after former delivery (months)	-	1.4 ± 0.3	0.9 ± 0.1	1.1 ± 0.3	1.2 ± 0.5	1.3 ± 0.6	1.6 ± 1.0	1.4 ± 1.0	1.6 ± 1.7	1.0 ± 0.2	0.8 ± 0.2	0.8 ± 0.0	1.2 ± 0.6

POTENTIAL CONFLICTS OF INTEREST. The authors declare that they have no conflict of interest.

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