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การเปรียบเทียบสมรรถภาพการสืบสายพันธุ์หนูเม้าส์เลือดชิด 3 สายพันธุ์ (BALB/cMlac, C57BL/6Mlac และ DBA/2Mlac) ของศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล ประเทศไทย

Reproductive Performance Comparisons of Three Inbred Mice Strains (BALB/cMlac, C57BL/6Mlac and DBA/2Mlac) at National Laboratory Animal Center, Mahidol University, Thailand

พรรัตนา ช่อมณี[1], อภิสิทธิ์ เหล่าสันติสุข[1], วสันต์ แก้วมณี[1], กาญจนา เข่งคุ้ม[1] และธนพร พิณพาทย์[1]*

Pornrattana Chumanee[1], Apisit Laosantisuk[1], Wanson Keawmanee[1], Kanchana Kengkoom[1] and Thanaporn Pinpart[1]*

บทคัดย่อ

สมรรถภาพการสืบสายพันธุ์อันประกอบด้วย ขนาดครอกโดยเฉลี่ย (mean litter size) อัตราส่วนการหย่านมต่อจำนวนลูกที่เกิด (wean: born ratio) และค่าดัชนีการสืบสายพันธุ์โดยเฉลี่ย (mean reproductive index) ของหนูเม้าส์เลือดชิดของศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดลจำนวน 3 สายพันธุ์ ได้แก่ BALB/cMlac C57BL/6Mlac และ DBA/2Mlac ได้ถูกคำนวณออกมาเพื่อเป็นข้อมูลพื้นฐานที่เกี่ยวกับการผสมและดูแลโคโลนีสืบสายพันธุ์ โดยหนูเม้าส์เลือดชิดทั้ง 3 สายพันธุ์นี้จัดอยู่ในสถานะสัตว์ปลอดเชื้อจำเพาะ (SPF) และได้รับการเพาะเลี้ยงอยู่ภายใต้ระบบการเลี้ยงแบบ Maximum barrier ที่ศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล การศึกษานี้ได้เก็บรวบรวมข้อมูลการสืบสายพันธุ์ของหนูเม้าส์เลือดชิดแต่ละสายพันธุ์ จำนวน 20 รุ่นการสืบสายพันธุ์ นำมาคำนวณและวิเคราะห์เปรียบเทียบผลทางสถิติด้วย t-test และ non-parametric test จากผลการคำนวณพบว่าขนาดครอก (แรกเกิด)โดยเฉลี่ยของหนู BALB/cMlac C57BL/6Mlac และ DBA/2Mlac เป็น 4.6±1 6.2±1.4 และ 4.3±1.0 ตามลำดับ และอัตราส่วนการหย่านมต่อจำนวนลูกที่เกิดโดยเฉลี่ยเป็น 0.90±0.1, 0.81±0.2 และ 0.83±0.2 ตามลำดับเช่นกัน นอกจากนั้นค่าดัชนีการสืบสายพันธุ์โดยเฉลี่ยของทั้ง 3 สายพันธุ์เป็น 1.03 0.94 และ 0.71 จากผลการเปรียบเทียบหนูเม้าส์เลือดชิดทั้ง 3 สายพันธุ์ได้บ่งชี้ว่าค่าสมรรถภาพการสืบสายพันธุ์ของหนู BALB/cMlac นั้นดีกว่าหนู C57BL/6Mlac และ DBA/2Mlac

คำสำคัญ: หนูเม้าส์/ สายพันธุ์ชิด/ สมรรถภาพการสืบสายพันธุ์/ ศูนย์สัตว์ทดลองแห่งชาติ

Abstract

Reproductive performance consisting of mean litter size, number of pups that wean (wean: born ratio) and mean reproductive index of three inbred mice strains, which are BALB/cMlac, C57BL/6Mlac and DBA/2Mlac, were investigated to provide some basic information on the breeding and maintaining of a foundation colony. These strains are designated as specific pathogen free animals (SPF) by the National Laboratory Animal Center, Mahidol University (NLAC-MU), the organization in which they were housed under the maximum barrier system. The data from twenty generations of each strain were analyzed by t-test and non-parametric test. The mean litter size (born) values were 4.6±1, 6.2±1.4 and 4.3±1.0, and the wean:born ratios were 0.90±0.1, 0.81±0.2 and 0.83±0.2 for BALB/cMlac, C57BL/6Mlac and DBA/2Mlac, respectively. Furthermore, their mean reproductive indexes were 1.03, 0.94 and 0.71, respectively. Comparison results of the three inbred strains suggested that the reproductive performance of BALB/cMlac is better than that of C57BL/6Mlac and DBA/2Mlac.

Keywords: Mice/ Inbred strains/ Reproductive performance/ NLAC-MU

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[1] Office of Laboratory Animal Production, National Laboratory Animal Center, Mahidol University, Nakhonprathom, 73170, Thailand

[1] สำนักงานผลิตสัตว์ทดลอง ศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล จังหวัดนครปฐม ประเทศไทย

*Corresponding author: thanaporn.pin@mahidol.ac.th

1. Introduction

The first inbred strains of mice were developed soon after the rediscovery of Mendel's laws of inheritance in 1900. More than 450 inbred mice strains have been described [1]. Genetically defined inbred strains make them more stable, more uniform, more repeatable, and better defined than the genetically undefined outbred stock [2]. Thus, inbred mice became essential animal models for scientific research during the 20th century and will have a decisive impact in the current and next centuries [3], especially the main inbred mouse strains, such as BALB/c, C57BL/6 and DBA/2. The BALB/c strain has been used broadly for general purposes that include hybridoma development, monoclonal antibody production, infected disease and oncological research [4]. In terms of C57BL/6, this strain has been used widely as a physiological or pathological model. Moreover, this strain is often used as a background strain for the generation of a transgenic or/and congenics model [5]. For DBA/2, this strain is also widely used in biomedical research, especially in the study of cardiovascular disease and neurobiology [6-7]. In Thailand, the National Laboratory Animal Center, Mahidol University (NLAC-MU), is an organization which has a mission to produce high quality laboratory animals for research purposes. A colony of inbred mice at NLAC-MU was established in 1999. All inbred mice strains produced at NLAC-MU, which are foundation stock, have been housed under the specific pathogen free status and have been maintained by sibling mating. Thus, the aim of this study is to describe and to compare the reproductive performance of three inbred mice strains (BALB/cMlac, C57BL/6Mlac and DBA/2Mlac) at NLAC to provide some basic information for breeding and research targets.

2. Materials and methods

Inbred mice and breeding

The origins of the three inbred mice strains (BALB/cMlac, C57BL/6Mlac and DBA/2Mlac) that are housed at NLAC-MU were obtained from the Central Institute for Experimental Animals (CIEA), Japan, in 1999. They have been set up as the foundation stock and kept under the maximum barrier, which is defined as a specific pathogen free status. Inbred lines have been maintained by sister-brother mating. All breeders have been mated permanently as monogamous pairs. The breeding performance of every female, including date of pups' birth, total number born per litter, number born alive, number of males, number of females and weaning date, was recorded carefully into the pedigree chart. Temperature and humidity in the foundation stock room were controlled at 22±2°C and 50 to 70% relative humidity, respectively. The light and dark cycle was 12:12 hour. Food and hyperchlorinated water (10-12 ppm.) were provided *ad libitum*.

Figure 1. Three inbred mice strains(BALB/cMlac, C57BL/6Mlac and DBA/2Mlac) housed at NLAC-MU.

Data analysis

A statistical analysis of the productivity data was carried out after the pooling of some data for a simplification analysis. Raw data on breeding performance from twenty generations of each strain were analyzed. The number of dams for BALB/cMlac, C57BL/6Mlac and DBA/2Malc were 115, 118 and 112 respectively. However, the dams which produced less than two litters were excluded in this analysis. Data to be analyzed consists of total number of litters, total number of pups born, total number of pups died and total number of pups weaned. Then, each reproductive performance characteristic derived from these data was calculated. These calculations generated mean litter size, wean:born ratio and mean of young weaned per female per week (mean reproductive index). Note that the reproductive index of each dam was calculated as follows:

$$\text{Reproductive index (RI)} = \frac{\text{total number of weaned} \times 7}{\text{mating period}}$$

A mating period starts from the first mating date to the weaning date of the last litter. Statistical analyses were performed using SPSS Statistics 18.0. The Kolmogorov-Smimov test was used to test the normality of data. Statistical comparisons among groups were done using one-way ANOVA and post hoc tests (LSD) for normally distributed data. The Mann-Whitney or Kruskal Wallis test was used for non-normally distributed data. A level of $P<0.05$ was accepted as statistically significant.

3. Results and discussion

Reproductive performance of three inbred strains

The total number of pups born from twenty generations of BALB/cMlac, C57BL/6Mlac and DBA/2Mlac were 1,353, 1,596 and 1,215, respectively. The total number of pups weaned was 1,232, 1,305 and 1,031, respectively. The mean (±SD) of characteristics, including mean litter size (born), wean:born ratio, mean reproductive index (mean RI) and statistical results of three inbred mice strains, are summarized in Table 1.

Table 1 Mean (±SD) of reproductive characteristic of three inbred mice strains of NLAC-MU.

Strain	Total number of dam	Weaning age (week)	Mean litter size (born)	Wean : Born ratio	RI
BALB/cMlac	52	3	4.6[a] ±1.0	0.90[a]±0.1	1.03
C57BL/6Mlac	56	3	6.2[a]±1.4	0.81±0.2	0.94[b]
DBA/2Mlac	61	3	4.3±1.0	0.83±0.2	0.71[c]

[a] Value is significantly different ($P<0.05$) between two other strains. [b] Value is significantly different ($P<0.05$) between C57BL/6Mlac and DBA/2Mlac. [c] Value is significantly different ($P<0.05$) between BALB/cMlac and DBA/2Mlac.

From Table 1, the mean litter size of BALB/cMlac, C57BL/6Mlac and DBA/2Mlac were 4.6, 6.2 and 4.3, respectively. It shows that C57BL/6Mlac litter size is bigger than that of BALB/cMlac and DBA/2Mlac. This is similar to the findings that C57BL/6 has excellent reproduction. Ranges of pups born were informed in several ranges, while mean litter size was reported in a range of 6 to 8 pups [8]. Similarly, BALB/c is also regarded as a common inbred strain that has a good reproductive performance [9]. Ranges of pup born of BALB/c were reported as 2 to 11 pups and 8 to 14 pups [10-11]. Furthermore, mean litter size was reported as 5 to 7.2 [12-13]. On the other hand, DBA/2 has a poor reproductive performance, and its mean litter size was reported as 3 to 4 [14].

Wean:born ratio and mean RI of three inbred mice strains in Table 1 indicate that values of BALB/cMlac are higher than those of C57BL/6Mlac and DBA/2Mlac. This shows that the BALB/c mouse has good behaviors in nursing their pups, and are better than those of the two other strains. Although the C57BL/6 mouse would produce a higher number of pups per litter, it tends to be a poor mother. For example, it is aggressive,

cannibalistic and/or it sometimes fails to provide appropriate care which leads to infant death due to hypothermia or starvation [15]. In terms of DBA/2Mlac, Table 1 indicates that it has the lowest reproductive performance. This is consistent with the summary of the biological characteristics report of the DBA/2 mouse, which is available from the National Institute on Aging [16].

Finally, in addition to behavior and genetic factors, reproduction and reproductive performance of inbred mice may be influenced by various environmental factors. In any case, significant differences in mean litter size, wean: born ratio and RI between inbred mice strains (Table 1) may be caused by many environmental factors, such as nutrition, drinking water, nesting materials, enrichments,etc. [17-18]. Therefore, physical environment is very important for maintaining the health and welfare of a breeding colony, which leads to productivity and success.

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