การคัดเลือกและจัดตั้งโคโลนีหนูแรท Mlac:WR ที่มีอุบัติการณ์ไตบวมน้ำต่ำ ที่ศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล ประเทศไทย

Selecting and Establishment of a Low-Incidence Hydronephrosis Wistar Rat (Mlac:WR) Colony at the National Laboratory Animal Center, Mahidol University, Thailand

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อจัดตั้งโคโลนีหนูแรท Mlac:WR ที่มีอุบัติการณ์ไตบวมน้ำต่ำที่ศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล ประเทศไทย พ่อแม่พันธุ์หนูแรท Mlac:WR จำนวน 20 คู่ในโคโลนีตั้งต้นของศูนย์สัตว์ทดลองแห่งชาติถูกสุ่มขึ้นมาเพื่อใช้ในการตรวจหาเปอร์เซ็นต์การเกิดอุบัติการณ์ไตบวมน้ำ พบว่ามีเปอร์เซ็นต์การเกิดไตบวมน้ำอยู่ที่ 9.66% ของประชากร การคัดเลือกพ่อแม่พันธุ์เพื่อดำเนินการสืบสายพันธุ์นั้นใช้การคัดเลือกด้วยวิธีการคัดเลือกจากลักษณะของรุ่นลูก ร่วมกับหลักการสืบสายพันธุ์หลีกเลี่ยงการเกิดเลือดชิดมากที่สุด และระบบการผสมพันธุ์แบบหมุนวน คู่พ่อแม่พันธุ์ที่ให้ลูกที่มีลักษณะไตบวมน้ำถูกคัดออกจากโคโลนี และถูกทดแทนด้วยลูกที่มาจากคู่พ่อแม่พันธุ์อื่นที่มีสุขภาพดีในกลุ่มเดียวกันในรุ่นถัดไป ผลจากการคัดเลือกและสืบสายพันธุ์พบว่าอุบัติการณ์ไตบวมน้ำ มีค่า 7.5% ในรุ่นที่ 0 ลดลงเหลือ 1.07%-1.72% ในรุ่นที่ 2-4 และลดลงเป็น 0.00% ในรุ่นที่ 5 อย่างไรก็ตาม ได้พบอุบัติการณ์ไตบวมน้ำ มีค่า 0.49% ในรุ่นที่ 6-7 ลดลงเป็น 0.00% ในรุ่นที่ 8 และพบว่า มีค่า 0.42%-1.02% ในรุ่นที่ 9-10 ทั้งนี้เนื่องจากลักษณะไตบวมน้ำเป็นลักษณะที่เกี่ยวข้องกับพันธุกรรมและถูกควบคุมด้วยยีนหลายยีน ดังนั้นจึงไม่สามารถกำจัดออกจากโคโลนีได้อย่างสมบูรณ์

คำสำคัญ: หนูแรทสายพันธุ์ Wistar/ Mlac:WR/ ไตบวมน้ำ/ การคัดเลือก/ ศูนย์สัตว์ทดลองแห่งชาติ

Abstract

This study established a low incidence hydronephrosis Wistar rat (Mlac:WR) colony at the National Laboratory Animal Center, Mahidol University, Thailand. Twenty Mlac:WR breeding pairs were randomized to determine the percentage of hydronephrosis in the original National Laboratory Animal Center colony. Hydronephrosis was discovered in 9.66% of the population. Breeder selection for breeding was carried out by

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using the progeny selection method, which selects offspring based on their characteristics, in combination with the breeding principle of maximum avoidance of inbreeding and the rotational mating system. Breeding pairs that produced hydronephrosis offspring were removed from the colony and replaced with offspring from other healthy breeding pairs within the same group in the next generation. The results from selection and breeding revealed that the incidence of hydronephrosis was 7.5% in the F0, decreasing to 1.07%-1.72% in the F2-F4, and 0.00% in the F5. However, the incidence of hydronephrosis was found at 0.49% in the F6-F7, decreasing to 0.00% in the F8, and was found again at 0.42%-1.02% in the F9-F10. This is because hydronephrosis traits are genetically linked and controlled by several genes. Therefore, it cannot be completely eliminated from the population.

Keyword: Wistar rat/ Mlac:WR / Hydronephrosis/ Selection/ National Laboratory Animal Center

1. Introduction

Hydronephrosis (HN) is a condition in which the renal pelvis and calyces dilate as a result of a functional or mechanical problem with the upper end of the ureter or the kidney pelvis [1]. HN is occasionally found in animals such as dogs and cats [2], swine [3], and cattle [4]. In laboratory rats, spontaneous HN is a well-known occurrence. Since the 1960s, rats have been reported to have sporadic HN [5]. The incidence of spontaneous HN in rats has been reported in several strains and stocks. In Sprague-Dawley, the incidence of HN was reported in two stocks (gender not specified), 3.9% in Crl:COBS[R]CD[R](SD) and 13.9% in Hap: (SD) [6]. Similarly, in Wistar, the incidence of HN was reported at 13.9% and 4.6% for males and females respectively, 11.29% for gender not specific [7]. Moreover, in some stock, such as Wistar rats which developed by Friedman and colleagues, has been found to develop HN with high rate at 95% and 60% for males and females respectively [8]. The National Laboratory Animal Center, Mahidol University (NLAC-MU) in Thailand is a non-profit organization whose goal is to produce high-quality laboratory animals for research. NLAC-MU has its own outbred rat colony (Wistar rat) under the name Mlac:WR for use in the country's OECD-GLP testing service. NLAC-MU has a routine health monitoring program to ensure that laboratory animals meet the health quality standard. Mlac:WR rats were found to have the incidence of HN through the routine health monitoring program. This was a serious topic since HN has a number of factors that can affect health, well-being, and life expectancy, all of which may increase economic significance, interfere with experimental investigations, or lead to misinterpretation of results. Therefore, the aim of this study was to solve HN topic by selecting and breeding Mlac:WR in order to establish of a Mlac:WR colony with low HN at the NLAC-MU in Thailand. The assumption in this study was continuous selection for at least 10 generations could reduce the incidence of HN in Mlac:WR to less than 2%.

2. Materials and Methods

Outbred Rat and Breeding

An origin of the Wistar rat housed at the NLAC-MU, was obtained from Veterinary Surgeon Mollegaards Breeding Center Ltd., Denmark, in 1980. It has been set up as the foundation stock and housed under the low barrier with HVAC as monitor animal status. In 2009, the Mlac:WR was registered with the Institute for Laboratory Animal Research (ILAR) by NLAC-MU. This outbred line has been maintained by maximum avoidance of inbreeding, a mating strategy in which animals are mated with respect to unrelatedness as possible. All breeders were given temporary mating pairs with an identification number at 8-10 weeks old. The animal room's temperature and humidity were maintained at 22±3°C and 50 to 70% relative humidity, respectively. The light-dark cycle was 12:12 hours

long. Food and hyperchlorinated water (5-6 ppm.) were provided *ad libitum*.

Selection

Progeny selection, selection based on the phenotype of offspring, was used to select the mating pair. Any breeding pair that did not produce any offspring in that cycle were excluded from the colony. Due to HN was the trait that cannot be identified by appearance, all pups born in the first litter must be kept and sent for HN screening at the age of 5 weeks. All Mlac:WR rats were euthanized by CO_2 and death confirmed by observing vital signs, including lack of pulse, breathing, toe pinch and cardiac muscle movement. All kidneys were dissected for HN screening. The total number of Mlac:WR rats with HN were recorded and traced back to the breeding pairs. Any breeding pairs produced HN offspring were excluded from the colony. This selection is under Mlac:SD and Mlac:WR Animal Production protocol which has been approved by National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC). All the animals in this selection were bred and cared for according to the guide, Guide for the Care and Use of Laboratory Animals [9].

Data Analysis

Descriptive statistics was used to describe data obtained from screening for HN, that was, the percentage of HN in each generation.

3. Results and Discussion

Selection and Incidence of Hydronephrosis

Data was collected from a total of 11 generations of rats (F0-F10), giving a total of 2,445 rats. The rats, Mlac:WR, which were apparently healthy from the original colony, were randomly assigned to 20 breeding pairs, numbered 1 to 20, respectively. They were flagged as *'F0 breeder screening'* to determine the incidence of HN within the original colony and were the initial breeders (F0) in the selection. This selection was determined on whether either breeding pairs did not give any offspring or produced HN offspring, they were excluded from the colony. Data and the results of screening were shown in Table 1.

Table 1 Percentage of 5 weeks old Mlac:WR with hydronephrosis (HN) in original colony

Generation	No. of breeding pair	Total No. of pups born in first litter	No. of pups with HN	HN (%)
F0 breeder screening	20	207	20	9.66

After mating, there were 2 breeding pairs did not give any offspring. They were excluded from colony. From Table 1, the percentage of HN in the original Mlac:WR colony was approximately 9.66%. This finding indicated that the incidence of HN in Wistar rat colony at the NLAC-MU, was lower than previously reported by Burton which reported at 11.29% [7]. The incidence of HN at NLAC-MU was lower than the others, possibly due to NLAC-MU's veterinary care program and routine health monitoring program for rat health surveillance. These help rapidly detect the incidence of HN in the Mlac:WR colony. The results of the offspring screening revealed that *F0 breeder screening* had 8 of the 18 breeding pairs with HN offspring. These 8 breeding pairs with HN offspring were excluded from the colony. All of the remaining 10 pairings (10 males, 10 females) were assigned to breeders in the F0 initial.

The F0 initial breeders (10 breeding pairs) were divided into 5 units, each consisting of 2 breeding pairs. For HN trait screening in each sex of breeder, the breeding pairs in each unit were switched sex partners as shown in Figure 1. The total number of offspring in first litter, 120 pups, were sent to HN screening. It was detected in 9 of 120 pups,

representing 7.05 percent of the total. There were 3 breeding pairs in F0 initial with HN offspring. They were excluded from the colony, remaining 7 breeding pairs, which were assigned to the new 7 units (and gave new identification no.) which was called the F0.

The F0 breeders and subsequent generations were bred on the basis of maximum avoidance of inbreeding and rotational mating system [10]. The mating diagram was shown in Figure 2. After mating, all pups were kept for F1 breeders.

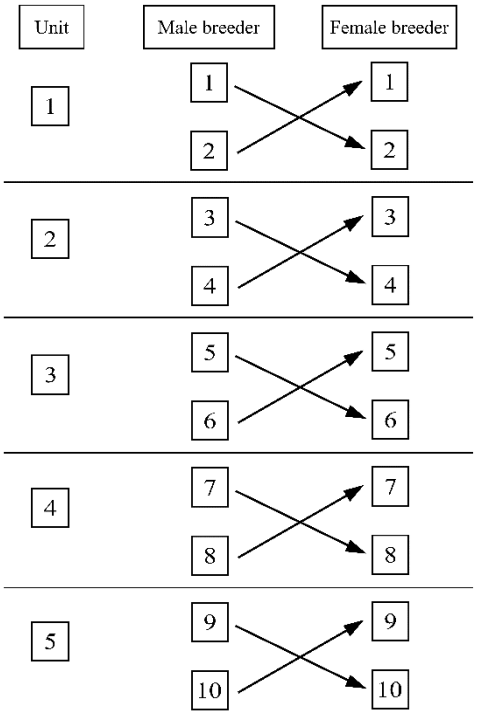

Figure 1 The F0 initial mating diagram, breeding pairs in each unit were switched sex partners. The bold arrows indicate the mating direction.

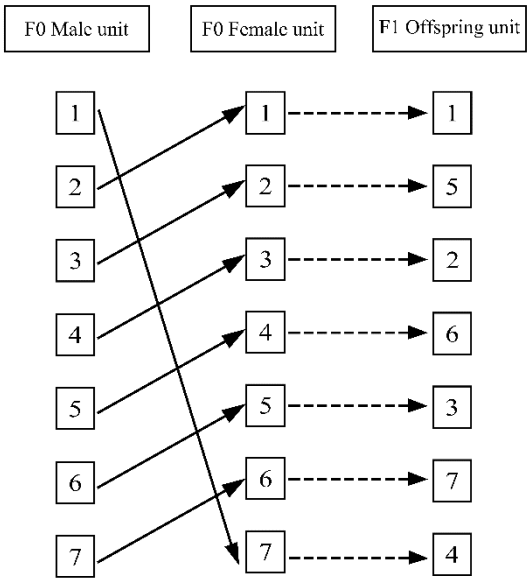

Figure 2 The F0 mating diagram. The bold arrows indicate the mating direction. The dotted arrows indicate the offspring.

The same as in F0's selection, F1 breeders, males and females were re-divided into 5 units, each consisting of 3 or 4 breeding pairs, total of 19 or 20 breeding pairs. All F1 breeders were assigned a unit identification number as shown in Figure 2. The F1 breeders and subsequent generations were bred to produce offspring in each unit as shown in Figure 3. Any breeding pair that did not produce any offspring after mating was eliminated. For selection, progeny selection was applied. Any breeding pairs giving HN offspring were excluded. To maintain the total number of breeding pairs at 20 or 5 unit, healthy offspring from other breeding pairs within the same unit were kept as replacement breeders for the next generation.

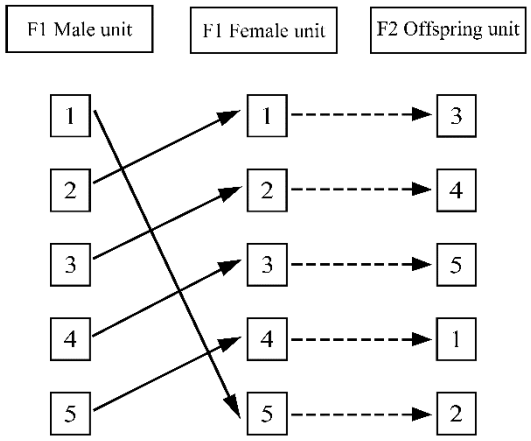

Figure 3 The F1 and subsequent generations mating diagram. The bold arrows indicate the mating direction. The dotted arrows indicate the offspring

In this selection, a total of 2,238 five-week-old pups (the first or second litter of F0-F10 breeders) were euthanized and necropsied for HN findings. The number of breeder pairs and screening results for HN in progeny were represented as percentage in Table 2.

Table 2 Percentage of 5 weeks old Mlac:WR with hydronephrosis (HN) in each generation

Generation	No. of breeding pairs	Total No. of pups born in first litter	No. of pups with HN	HN (%)
F0 initial	10	120	9	7.50
F1	19*	182	10	5.49
F2	20	187	2	1.07
F3	20	278	5	1.08
F4	20	232	4	1.72
F5	20	205	0	0.00
F6	20	206	1	0.49
F7	20	203	1	0.49
F8	20	189	0	0.00
F9	20	240	1	0.42
F10	20	196	2	1.02

*a female breeder died before mating, one pair of breeders was eliminated.

According to Table 2, the incidence of HN was 7.50% in the F0 generation and 5.49% in the F1 generation, respectively. HN was reduced dramatically after selection, with the incidence of HN in the F2-F4 ranges from 1.07%-1.72% and decreased to 0.00% in the F5. However, it was discovered that HN was still presented in subsequent generations (F6-F10), with an incidence of 0.00%-1.02%. The inability to completely separate HN from the colony because HN is genetically related. In Wistar-derived Gunn rats HN is a congenital condition that is inherited from an autosomal trait and as autosomal dominant lethal gene when homozygous [11,12]. In addition, a genetic study in the inbred rat strain, Brown Norway rat, indicated that bilateral HN was significantly linked to a locus on chromosome 6, while right-sided HN was linked to loci on chromosomes 2 and 4 [13].

Finally, when breeding follows the pattern shown in Figure 3 and is combined with continued breeder selection for subsequent generations, the incidence of HN in a colony can be kept to less than 2.00%. NLAC-MU was successful in establishing a low-incidence HN Wistar rat (Mlac:WR) colony. This is useful for colony management in the production of high-quality Mlac:WR rats for usage in many research fields. For future work, NLAC-MU will develop the low-incidence Mlac:WR rat into an inbred rat strain, the Free Hydronephrosis Wistar Rat.

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