BPB Reports

Report

Sperm Morphology is Different in Two Common Mouse Strains

Nao Sekine,[a,1] Satoshi Yokota,[a,b,1] and Shigeru Oshio*[,a]

[a]*Department of Hygiene Chemistry, Ohu University School of Pharmaceutical Sciences, 31-3 Misumido, Tomita, Koriyama, Fukushima 963-8611, Japan;* [b]*Division of Cellular & Molecular Toxicology, Center for Biological Safety & Research, National Institute of Health Sciences 3-25-26 Tono-machi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan*

Received June 3, 2021; Accepted August 27, 2021

ICR and C57BL/6J mice have been widely used in several research fields. The reproductive toxicology parameters, such as fertilization rate, which may differ between the two strains, are well known. However, the details of the sperm quality parameters are not well known. To reveal these, we compared the sperm morphology of the two strains. Eosin-stained sperm smears from adult ICR and C57BL/6J mice were analyzed. We observed that 79.6 ± 1.2 and 49.5 ± 1.7% of ICR and C57BL/6J mice sperm, respectively, showed a normal form. Furthermore, abnormal sperm samples were classified into ten types based on their defective sites. The percentage of abnormal sperm with an amorphous head, bent head, no head, hairpin loop, short tail, and two tails in ICR mice was significantly lower than that in C57BL/6J mice. In contrast, the percentage of coil-tailed sperm in ICR mice was significantly higher than that in C57BL/6J mice. These results suggest that C57BL/6J mice have a limited ability to remove the cytoplasm during spermiation and ICR mice have fewer sperm abnormalities than C57BL/6J mice. The characteristics of male reproductive traits among mouse strains should be taken into consideration in sperm analysis, as the negligence of this could generate an increased potential for a misleading in toxicology evaluation.

Key words ICR, C57BL/6J, sperm morphology, spermatogenesis

INTRODUCTION

Toxicology studies in reproduction are designed to examine the effects of exposure to chemical substances on the reproductive function and development of the offspring. Sperm quality parameters, such as sperm count, motility, morphology, and viability are generally used.[1)] Multilateral evaluation of these parameters is good for elucidating testicular toxicity caused by chemical exposure.

ICR mice have superior characteristics including rapid growth, strong constitution, very docile nature, and fairly good reproductive ability (average litter size 13.6).[2)] This strain generally has very little variability among the inheritable characteristics.[3)] Therefore, genetic traits are maintained at a constant level when closed colony mice are observed in a group. In contrast, C57BL/6J mice are commonly used to conduct behavioral studies because they are lively and capable of learning a variety of tasks.[4)] In addition, C57BL/6J mice have low variability, which enables the analysis of reproducibility through genetic research.[3,4)] However, this strain has relatively lower reproductive ability (average litter size 6.7)[2)] than ICR. Sperm-related parameters of both strains differ,[5)] however, differences in the proportions of various types of anomalies in sperm morphology have not yet been evaluated.

In the present study, we aimed to estimate the general morphological anomalies in the spermatozoa of ICR and C57BL/6J mice.

MATERIALS AND METHODS

Animals and Tissue Sampling Male ICR and C57BL/6J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Both groups were bred from 3 to 10 weeks of age in home cages at 23 ± 2°C on a 12 h light/dark cycle (lights on from 08:00 to 20:00). Food and water were provided ad libitum during the experiment. After intraperitoneal injection of pentobarbital at 10 weeks of age, we collected the reproductive organs. All efforts were made to minimize suffering. Dissected tissues, except for the epididymis, were rapidly frozen in liquid nitrogen and stored at −80°C.

All experiments were performed following the National Institute of Health (USA) guidelines for animal experiments and were approved by the Animal Care Committee of Ohu University (approval no. 2016-40).

Analysis of Sperm Quality This method was performed as previously reported.[6)] The isolated cauda epididymis was minced with small scissors in 1 mL of 10 mM HEPES-TYH culture medium in a sterile 1.5-mL tube. Sperm suspensions were allowed to disperse for 15 min on a warming tray at 37°C. These suspensions were then filtered using a high-quality 40 μm nylon mesh (PP-40N, Kyoshin Rikoh Inc., Tokyo, Japan) to remove any undigested tissue fragments, and the sperm was collected for count, motility, and viability evaluation.

Sperm count, viability, and motility[6)] were analyzed using a phase-contrast microscope (BX51, Olympus Co., Tokyo, Japan). Sperm motility was analyzed using a Sperm Class Analyzer

*To whom correspondence should be addressed. e-mail: s-oshio@pha.ohu-u.ac.jp
[1] These authors contributed equally to the work.

(Microscopic SL, Barcelona, Spain) and the progressive motile sperms were detected based on the movement of at least 500 sperm cells. Sperm cells were recorded in at least four random fields for each sample. Sperm viability was measured by mixing the sperm suspension with eosin Y solution (1%).

Daily sperm production (DSP) was also measured.[6,7] Briefly, half of the right testes were dissected and homogenized using a Polytron homogenizer (PT 1300D Homogenizer; KINEMATICA AG, Luzern, Switzerland) in homogenization buffer containing saline, 0.05% Triton X-100 (Nacalai Tesque, Inc., Kyoto, Japan), and 0.2% eosin Y (Merck & Co., Inc., Kenilworth, NJ, USA). The concentration of sperm nuclei in each suspension was determined using a hemocytometer under a light microscope. The counts served as a basis for the calculation of the number of homogenization-resistant spermatids per gram of testicular tissue.

Evaluation of Morphological Anomalies of Sperm Cells One drop of the sperm suspension was placed on a glass slide and spread using a coverslip. Sperm cells were fixed in an ethanol-acetic acid, followed by staining with 1% eosin Y solution. The samples were air-dried before morphological observation. Sperm morphology was examined using sperm smear preparations under a microscope, and the percentage of sperm cells with a normal form was calculated and classified depending on the site of abnormality, and at least 200 spermatozoa were analyzed per mouse. In particular, 11 types of morphological classifications were evaluated as follows: Normal morphology, amorphous head, bent head, no head, two heads, small head, round head, hairpin loop, coil tail, short tail, and two tails. This classification method was based on previous studies.[8–12]

Statistics Unless otherwise noted, data are presented as the mean ± standard error. Statistical analyses were performed using the Wilcoxon rank-sum test. Significance was set at $P < 0.05$.

RESULTS

Mouse Growth and Development The body weight (ICR: 40.1 ± 1.0 g vs C57BL/6J: 29.3 ± 0.8 g) and testicular weight (ICR: 0.13 ± 0.004 g vs C57BL/6J: 0.10 ± 0.002 g) of ICR mice were significantly higher than those of C57BL/6J mice ($P < 0.001$). However, we observed no difference in both testis weight/body weight ratio and histological examination of the seminiferous tubule area between ICR and C57BL/6J mice (data not shown).

Functional Parameters of Sperm Cells The DSP per gram of testicular tissue of ICR mice was significantly lower than that of C57BL/6J mice ($P = 0.011$) (Fig. 1). No significant differences in sperm motility and viability were found between ICR and C57BL/6J mice.

Sperm Morphological Analysis We observed that 79.6 ± 1.2% of epididymal sperm in ICR mice showed a normal form, compared with 49.5 ± 1.7% in C57BL/6J mice (Fig. 1). There was a significant difference in the number of morphologically normal sperms ($P < 0.001$) between ICR and C57BL/6J mice; differences in the proportions of various types of anomalies were also observed. Briefly, the percentage of abnormal sperm with amorphous head, bent head, no head, hairpin loop, short tail, and two tails in ICR mice was significantly lower than that in C57BL/6J mice ($P < 0.001$) (Table 1). In contrast, the percentage of coil-tailed sperm in ICR mice (9.2 ± 1.1%) was significantly higher than that in C57BL/6J mice (5.4 ± 0.6%) ($P < 0.001$) (Table 1).

DISCUSSION

The purpose of this study was to evaluate the properties of spermatogenesis in ICR and C57BL/6J mice using sperm analysis. DSP in ICR mice was significantly lower than that in C57BL/6J mice. However, the body weight and testicular weight of C57BL/6J mice were significantly lower than those of ICR mice. Sperm analysis is routinely used as a clinical test to diagnose male infertility because of its non-invasiveness and simplicity.[13] In animals, epididymal sperm (from rodents) or ejaculated sperm (from rabbit, dog, monkey, etc.) are also analyzed as indicators of fertility.[14] Rodents are used as a model of male fertility and are also key to study reproductive toxicity.[15]

Our results showed that the testis size of C57BL/6J mice was small, although C57BL/6J mice had a high rate of sperm production. Sperm motility and viability were not substantially different between ICR and C57BL/6J mice; thus, no characteristic differences in the sperm motility parameters regarding male fertility were found between them. However, the number of morphologically normal sperm in ICR mice was significantly higher than that in C57BL/6J mice. Previous studies on sperm morphology were reported that normal morphological sperm % in ICR mice were around 70 to 80%,[16–18] in contrast, those in C57BL were around 50%.[19,20] These results agree well with the present study. Albert and Russel[21] reported that the strain differences in the concentration, sperm motility and morphology of epididymal sperm. They indicated that % of sperm motility of BALB, CBA, C3H and C57BL of mice were 54.5, 53, 53, 54.5, however, % of normal morphological sperm of these four strains were 42.4, 62.6, 56.8, 48, respectively. These results suggest that sperm motility does not always correlate with normal morphology. In ICR mice, the percentage of the sperm cells with an abnormal tail was higher than that of those with an abnormal head. In C57BL/6J mice, the percentage of sperm cells with an abnormal head was higher than that of those with an abnormal tail. These findings suggested that there is a difference in normal spermatogenesis and the frequency of morphologically abnormal sperm production between ICR and C57BL/6J mice. Therefore, differences in the proportions of various types of anomalies were observed to compare the morphological characteristics of these strains.

Abnormal sperm forms were classified into ten types based on defective sites as follows: Amorphous head, bent head, no head, small head, round head, and two heads. Among these morphological abnormalities, percentages of sperm cells with an amorphous head, bent head, or no head in C57BL/6J mice were significantly higher than those in ICR mice, and the amorphous head was the most frequently observed abnormality in C57BL/6J mice.

According to these results, the mechanism responsible for the increase in the number of sperm cells with an abnormal head was not due to a defect in the acrosome but a defect in the head/neck region. The amorphous head appeared as an inverted head. For instance, spermatid maturation 1 (spem1) -null sperm cells display deformation with heads bent backward pointing to tail tips or bent heads wrapped around necks and the middle of the tails.[22] The amorphous head is similar to the spem1-null sperm in morphology, and therefore, the appearance of an amorphous head is thought to be caused

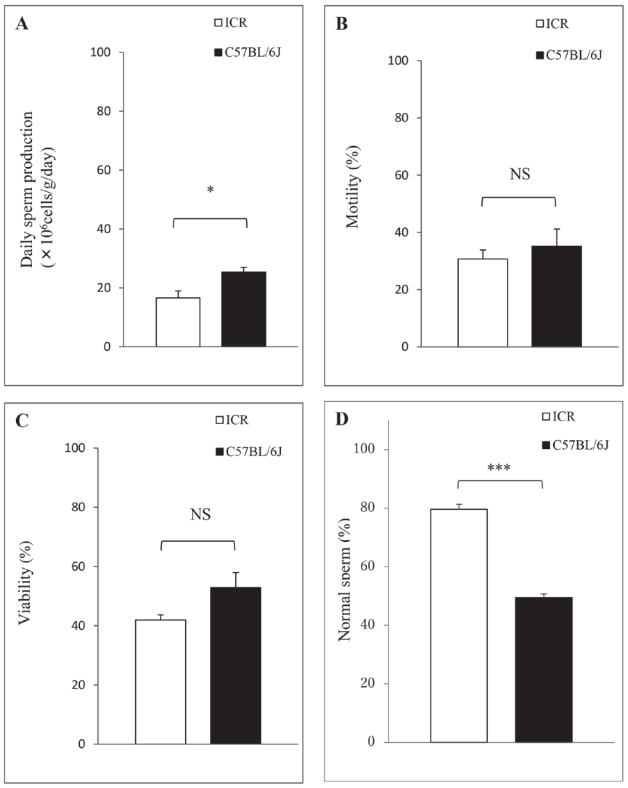

Fig. 1. Functional Parameters of Sperm Function

(A) Significantly low daily sperm production in ICR mice (16.6 ± 2.4%; n = 8) compared with C57BL/6JJcl mice (25.4 ± 1.6%; n = 10). Data are presented as the mean ± standard error (SE). (B) Sperm motility in ICR mice (30.7 ± 3.2%; n = 8) was not different from that in C57BL/6JJcl mice (35.2 ± 6.0%; n = 11). Data are presented as the mean ± SE. (C) Sperm viability in ICR mice (41.9 ± 1.8%; n = 8) was not different from that in C57BL/6JJcl mice (52.9 ± 5.1%; n = 10). Data are presented as the mean ± SE. (D) Normal sperm in ICR and C57BL/6JJcl mice at 79.6 ± 1.7% (n = 8) and 49.5 ± 1.2% (n = 11), respectively. Data are presented as the mean ± SE. *p < 0.05, **p < 0.01, ***p < 0.001, NS: not significant.

by the remaining cytoplasmic components. Abnormal sperm tail was denoted as a hairpin loop, coil tail, short tail, or two tails. Among these morphological abnormalities, percentages of sperm cells with a hairpin loop, short tail, or two tails in C57BL/6J mice were significantly higher than those in ICR mice. In particular, the hairpin loop was the second most commonly observed feature in the abnormal sperm of C57BL/6J mice. The hairpin loop appeared to be formed by a defect at the midpiece and principal piece junction. For instance, sperm defects in septin4 (sept4) mutant mice are associated with excess cytoplasm retention.[23] The hairpin loop is similar to the sept4 mutant sperm in morphology, and therefore, the hairpin loop is also thought to be caused by the remaining cytoplasmic components. According to our results, both the amorphous head and hairpin loop were associated with the retention of the excess cytoplasm. In contrast, the percentage of sperm cells with a coiled tail in ICR mice was significantly higher than that in C57BL/6J mice, and the coiled tail was the most frequently observed feature in the abnormal sperm of ICR mice. However, the total number of tail defects in ICR mice was significantly lower than that in C57BL/6J mice. In several studies, sperm flagellum anomalies using knockout mice or mutant mice displayed multiple spermatogenetic defects.[24–30] These studies suggest that major abnormal sperm, including the coiled tail, remain undiscovered in knockout mice or mutant

Table 1. Sperm Morphological Status of ICR and C57BL/6J

	ICR (n = 8)	C57BL/6J (n = 11)
head abnormality		
Total[a]	8.9 ± 0.8	34.8 ± 1.5
amorphous head[a]	1.0 ± 0.4	19.7 ± 1.8
bent head[a]	2.3 ± 0.4	5.2 ± 0.4
No head[a]	3.8 ± 0.6	8.0 ± 0.9
two heads	0.1 ± 0.1	0.2 ± 0.1
small head	1.4 ± 0.5	1.5 ± 0.3
round head	0.4 ± 0.2	0.1 ± 0.1
tail abnormality		
Total[a]	11.9 ± 1.3	19.6 ± 0.9
hairpin loop[a]	1.3 ± 0.4	10.4 ± 0.7
coil tail[a]	9.2 ± 1.1	5.4 ± 0.6
short tail[a]	1.4 ± 0.4	3.7 ± 0.5
two tails[a]	0	0.1 ± 0.0

Values are represented as the mean ± standard error.
a: differences are significant at $p < 0.01$.

mice. Thus, it appears that the coiled tail is affected by external factors rather than genetic factors.

In the present study, we showed that the percentage of morphologically normal and abnormal sperm, classified depending on the site of abnormality, differ substantially between ICR and C57BL/6J mice. Taken together, our results suggest the possibility that C57BL/6J mice sperm have a low ability to remove the cytoplasm, as the percentage of almost all types of sperm abnormalities observed in ICR mice were lower than that in C57BL/6J mice. Thus, we considered that the characteristics of male reproductive traits among mouse strains should be taken into consideration in sperm analysis. Differences in the number of morphologically normal sperms among mouse strains could generate an increased potential for a misleading in toxicology evaluation.

Acknowledgments This work was supported in part by JSPS KAKENHI Grant number 19K19424 to SY and Ohu University Special Research Grant for Pharmaceutical Research to SO.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Sally D. Perreaultand, Aida M. Cancel Significance of incorporating measures of sperm production and function into rat toxicology studies. *Reproduction*, **121**, 207–216 (2001).
2) CLEA Japan Inc. https://www.clea-japan.com/products/inbred/item_a0500
3) Chia R, Achilli F, Festing MF, Fisher EM. The origins and uses of mouse outbred stocks. *Nat. Genet.*, **37**, 1181–1186 (2005).
4) Mekada K, Abe K, Murakami A, Nakamura S, Nakata H, Moriwaki K, Obata Y, Yoshiki A. Genetic Differences among C57BL/6 Substrains. *Exp. Anim.*, **58**, 141–149 (2009).
5) Kawai Y, Hata T, Suzuki O, Matsuda J. The relationship between sperm morphology and *in vitro* fertilization ability in mice. *J. Reprod. Dev.*, **52**, 561–568 (2006).
6) Yokota S, Takuya Shirahata T, Yusa J, Sakurai Y, Ito H, Oshio S. Long-term dietary intake of excessive vitamin A impairs spermatogenesis in mice. *J. Toxicol. Sci.*, **44**, 257–271 (2019).
7) Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J. Reprod. Fertil.*,

54, 103–107 (1978).
8) Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW, Jr Letz G, Malling HV, Topham JC, Whorton MD. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. A report of the U.S. Environ-mental Protection Agency Gene-Tox Program. *Mutat. Res.*, **115**, 1–72 (1983).
9) Takeda N, Yoshinaga K, Furushima K, Takamune K, Li Z, Abe S, Aizawa S, Yamamura K. Viable offspring obtained from Prm1-deficient sperm in mice. *Sci. Rep.*, **6**, 27409 (2016).
10) White MA, Steffy B, Wiltshire T, Payseur BA. Genetic dissection of a key reproductive barrier between nascent species of house mice. *Genetics*, **189**, 289–304 (2011).
11) Shirley CR, Hayashi S, Mounsey S, Yanagimachi R, Meistrich ML. Abnormalities and reduced reproductive potential of sperm from Tnp1- and Tnp2-null double mutant mice. *Biol. Reprod.*, **71**, 1220–1229 (2004).
12) Shin SC, Kang YM, Jin YW, Kim HS. Relative morphological abnormalities of sperm in the caudal epididymis of high- and low-dose-rate gamma-irradiated ICR mice. *J. Radiat. Res. (Tokyo)*, **50**, 261–266 (2009).
13) Behre HM, Yeung CH, Holstein AF, Weinbauer GF, Gassner P, Nieschlag E. Diagnosis of Male Infertility and Hypogonadism. Andrology. (Nieschlag E., Behre H.M. eds) Springer, Berlin, Heidelberg. pp.90-124 (2001)
14) Seidel GE Jr. Several insights on evaluation of semen. *Anim. Reprod.*, **9**, 329–332 (2012).
15) Auger J, Eustache F, Rouiller-Fabre V, Canivenc-Lavier MC, Livera G. Integrative rodent models for assessing male reproductive toxicity of environmental endocrine active substances. *Asian J. Androl.*, **16**, 60–70 (2014).
16) Oliveira H, Spanò M, Guevara MA, Santos TM, Santos C, Pereira Mde L. Evaluation of *in vivo* reproductive toxicity of potassium chromate in male mice. *Exp. Toxicol. Pathol.*, **62**, 391–404 (2010).
17) Shin SC, Kang YM, Jin YW, Kim HS. Relative morphological abnormalities of sperm in the caudal epididymis of high- and low-dose-rate gamma-irradiated ICR mice. *J. Radiat. Res. (Tokyo)*, **50**, 261–266 (2009).
18) Wu Y, Chen X, Wang S, Jiang M, Zheng B, Zhou Q, Bi Y, Zhou Z, Huang X, Sha J. Flotillin-2 is an acrosome-related protein involved in mouse spermiogenesis. *J. Biomed. Res.*, **26**, 278–287 (2012).
19) Bruner-Tran KL, Ding T, Yeoman KB, Archibong A, Arosh JA, Osteen KG. Developmental exposure of mice to dioxin promotes transgenerational testicular inflammation and an increased risk of preterm birth in unexposed mating partners. *PLoS One*, **9**, e105084 (2014).
20) Sohara E, Ueda O, Tachibe T, Hani T, Jishage K, Rai T, Sasaki S, Uchida S. Morphologic and functional analysis of sperm and testes in Aquaporin 7 knockout mice. *Fertil. Steril.*, **87**, 671–676 (2007).
21) Albert M, Roussel C. Strain differences in the concentration, motility and morphology of epididymal sperm in relation to puberty in mice. *Int. J. Androl.*, **7**, 334–347 (1984).
22) Zheng H, Stratton CJ, Morozumi K, Jin J, Yanagimachi R, Yan W. Lack of Spem1 causes aberrant cytoplasm removal, sperm deformation, and male infertility. *Proc. Natl. Acad. Sci. USA*, **104**, 6852–6857 (2007).
23) Kissel H, Georgescu MM, Larisch S, Manova K, Hunnicutt GR, Steller H. The sept4 Septin locus is required for sperm terminal differentiation in mice. *Dev. Cell*, **8**, 353–364 (2005).
24) Dong FN, Amiri-Yekta A, Martinez G, Saut A, Tek J, Stouvenel L, Lorès P, Karaouzène T, Thierry-Mieg N, Satre V, Brouillet S, Daneshipour A, Hosseini SH, Bonhivers M, Gourabi H, Dulioust E, Arnoult C, Touré A, Ray PF, Zhao H, Coutton C. Absence of CFAP69 causes male infertility due to multiple morphological abnormalities of the flagella in human and mouse. *Am. J. Hum. Genet.*, **102**, 636–648 (2018).
25) Coutton C, Martinez G, Kherraf ZE, Amiri-Yekta A, Boguenet M, Saut A, He X, Zhang F, Cristou-Kent M, Escoffier J, Bidart M, Satre V, Conne B, Fourati Ben Mustapha S, Halouani L, Marrakchi O, Makni M, Latrous H, Kharouf M, Pernet-Gallay K, Bonhivers M, Hennebicq S, Rives N, Dulioust E, Touré A, Gourabi H, Cao Y, Zouari R, Hosseini SH, Nef S, Thierry-Mieg N, Arnoult C, Ray PF. Bi-allelic mutations in ARMC2 lead to severe astheno-teratozoospermia due to sperm flagellum malformations in humans and mice. *Am. J. Hum. Genet.*, **104**, 331–340 (2019).
26) Wang W, Tu C, Nie H, Meng L, Li Y, Yuan S, Zhang Q, Du J, Wang J, Gong F, Fan L, Lu GX, Lin G, Tan YQ. Biallelic mutations in CFAP65 lead to severe asthenoteratospermia due to acrosome hypoplasia and flagellum malformations. *J. Med. Genet.*, **56**, 750–757 (2019).
27) Cheng Y, Buffone MG, Kouadio M, Goodheart M, Page DC, Gerton GL, Davidson I, Wang PJ. Abnormal sperm in mice lacking the Taf7l gene. *Mol. Cell. Biol.*, **27**, 2582–2589 (2007).
28) Abbasi F, Miyata H, Shimada K, Morohoshi A, Nozawa K, Matsumura T, Xu Z, Pratiwi P, Ikawa M. RSPH6A is required for sperm flagellum formation and male fertility in mice. *J. Cell Sci.*, **131**, jcs221648 (2018).
29) Ray PF, Toure A, Metzler-Guillemain C, Mitchell MJ, Arnoult C, Coutton C. Genetic abnormalities leading to qualitative defects of sperm morphology or function. *Clin. Genet.*, **91**, 217–232 (2017).
30) Sironen A, Shoemark A, Patel M, Loebinger MR, Mitchison HM. Sperm defects in primary ciliary dyskinesia and related causes of male infertility. *Cell. Mol. Life Sci.*, **77**, 2029–2048 (2020).