The Journal of Veterinary Medical Science

NOTE

Anatomy

Histological analysis of implanted embryos in large Japanese field mouse (*Apodemus speciosus*) and estimation of developmental stage

Hiroyuki IMAI[1)]*, Kiyoshi KANO[2)] and Ken Takeshi KUSAKABE[1)]

[1)]Laboratory of Veterinary Anatomy, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan

[2)]Laboratory of Veterinary Embryology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan

J. Vet. Med. Sci.
83(8): 1178–1181, 2021
doi: 10.1292/jvms.21-0197

Received: 30 March 2021
Accepted: 3 June 2021
Advanced Epub:
21 June 2021

ABSTRACT. The large Japanese field mouse (*Apodemus speciosus*) is a small rodent species endemic to Japan. The genetic characteristics of *A. speciosus* include different chromosome numbers within the same species. Furthermore, *A. speciosus* has been used in radiation and genetic research. In the present study, a pregnant *A. speciosus* was obtained, and histochemical analysis of the implanted embryos was performed and compared with the developmental stages of the mouse (*Mus musculus*). Although there were some differences, the structures of the implanted embryos, including the primitive streak and placenta of *A. speciosus* were similar to those of mouse. Our study will be important for the construction of a developmental atlas of *A. speciosus*.

KEY WORDS: *Apodemus speciosus*, embryo, histology, implantation, Large Japanese field mouse

The large Japanese field mouse (*Apodemus speciosus*) is a small rodent that inhabits Japan and it has diverged from its mouse and rat ancestors 1.5–4.4 hundred thousand years ago [7]. *A. speciosus*, found across Japan except for Okinawa, is used as a model for studying the geographic isolation of animals [16]. In addition, *A. speciosus* has distinctive genetic characteristics in that the number of chromosomes differs between those living in western and eastern Japan. This karyotypic alteration in *A. speciosus* was caused by a Robertsonian translocation [15]. In recent years, *A. speciosus* has been used as an animal model to monitor the effects of radiation from nuclear power plant accidents [3].

During the genetic analysis of *A. speciosus*, which is our current project, a pregnant *A. speciosus* was obtained by chance. With the permission, *A. speciosus* was captured using the sharman traps (LFA; Hoga, Kyoto, Japan). The pregnant *A. speciosus* was captured at Akiyoshidai, Mine City, Yamaguchi Prefecture, on October 26, 2020, and it weighted 38.22 g (Fig. 1A). The species of the mouse was determined from appearance and hindfoot length [1, 6] and confirmed by chromosome number and sequences in the mitochondrial genome (accession number: LC603620 and LC603624). The morphology of the implantation sites is compared with Theiler's stage and the atlas of development [8, 17] and summarized as a case report.

The uterine horns were divided into right and left sides, and implantation sites were numbered from the ovarian side (Fig. 1B). Six implantation sites and two implantation marks were identified. These implantation sites were fixed with Bouin solution and embedded

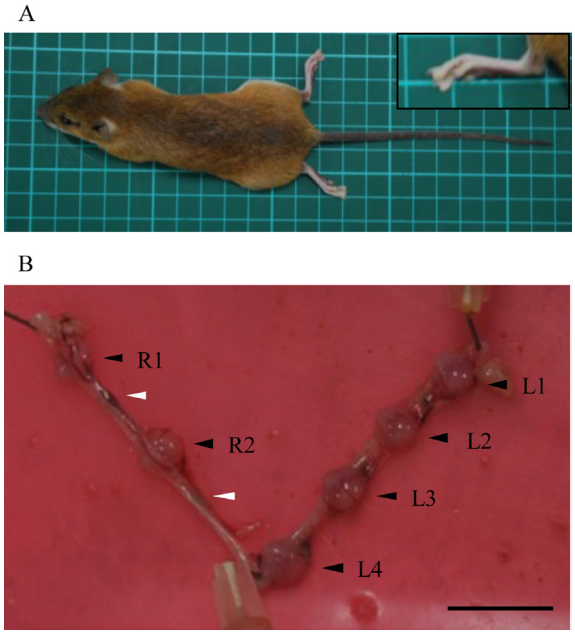

Fig. 1. Appearance of the pregnant *Apodemus speciosus* and the uterus. (A) Captured pregnant *A. speciosus*. An enlarged image of the hindfoot is shown in the upper right panel. Grid: 10 × 10 mm. (B) The implantation uterus of *A. speciosus*. The black arrowheads indicate the implantation site and the white arrowheads the implantation marks. Bar: 10 mm.

*Correspondence to: Imai, H.: himai@yamaguchi-u.ac.jp

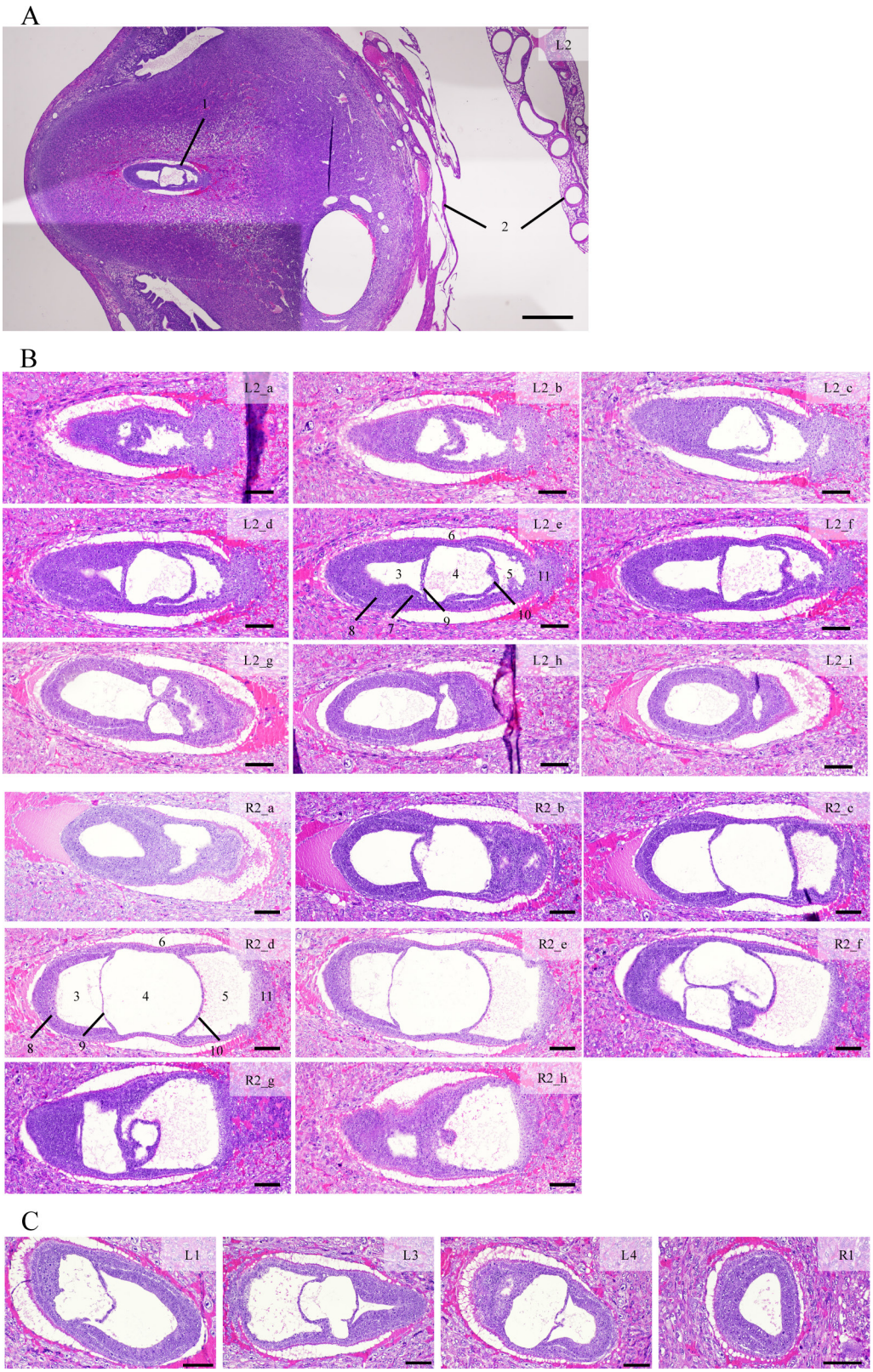

Fig. 2. Histological images of implantation sites in the *Apodemus speciosus*. **(A)** Lower magnification image of the L2 implantation site. 1. Implanted embryo and 2. mesometrium. Bar: 500 µm. **(B)** Serial images of the L2 and R2 implantation sites. 3. Amniotic cavity, 4. exocoelomic cavity, 5. ectoplacental cavity, 6. yolk sac cavity, 7. primitive streak, 8. epiblast, 9. amnion, 10. chorion and 11. ectoplacental cone. Bar: 100 µm. **(C)** Images of implantation site (L1, L3, L4 and R1). Bar: 100 µm.

in paraffin. Serial sections (5-µm-thick) were prepared using a rotary microtome (RM2245; Leica, Wetzlar, Germany) and stained with hematoxylin and eosin.

The position of the mesometrium in the implanted embryos indicated eccentric implantation, which is typical for rodents (Fig. 2A). Serial observation revealed typical epiblasts and three cavities (Fig. 2B). The primitive streak-like structures were similar to those of mouse embryos and consisted of three layers of cells, suggesting that the primitive streak-like structures containing these three cell layers were the primitive streak and epiblast of *A. speciosus* (Fig. 2B; 8 in L2_e, R2_d). There is a region that binds the maternal decidua and conception on the opposite side of the primitive streak, which is assumed to be the ectoplacental cone (Fig. 2B; 11 in L2_e, R2_d). Based on the orientation of the primitive streak and placenta, the three cavities in the conception appeared to be amniotic, ectoplacental and exocoelomic cavity, respectively (Fig. 2B; 3, 4 and 5 in L2_e, R2_d, respectively). The two membrane structures that separate these three cavities could be the amnion and the chorion (Fig. 2B, 9 and 10 in L2_e, R2_d). The same structures were observed at the L3 and L4 implantation sites (Fig. 2C). In L1 preparation, where only two cavities were found, part of the partition between the ectoplacental and exocoelomic cavities could be observed. For R1 preparation, where only one cavity was found, this might be a transverse section of the epiblast region, as there were three layers of cellular structures. Thus, these results indicated that *A. speciosus* embryos used in the present study were 7.5–8.0 day-old, which corresponds to stage 11. However, there were some features that did not completely correspond to the Theiler's developmental stages of mouse embryo; these features were as follows: 1. allantois, which is a stage-specific structure in exocoelom, was not clearly observed; and 2. no clear blood islets were observed. These structures could not be functionally deficient because they have important roles in embryonic development such as placentation and hematopoiesis. In golden hamsters with a gestation period of 15–18 days, allantois was observed in 8.0–9.0 day-old embryos [2, 5], suggesting that the timing of formation varies even within rodents. The developmental stage at which these structures are formed in *A. speciosus* might be different from that in mouse embryos. Therefore, *A. speciosus* embryos are morphologically similar to mouse embryos, but they also have subtle differences.

The present study analyzed the embryonic morphology in stage 11 of *A. speciosus* embryonic development. Although the ages of the embryos were unknown, the gestation period of *A. speciosus* is 21–26 days [12], these embryos in the present study might therefore be 7.9–10.4 day-old. Methods for rearing and breeding the *A. speciosus* have been developed previously [14], and an atlas of embryonic development of *A. speciosus* should be created in the future. Furthermore, the whole genome sequence of *A. speciosus* has already been published [10]. Therefore, *A. speciosus* can be established as a novel experimental rodent with the following characteristics: 1. rodents with seasonality in spermatogenesis and reproduction [9, 13]; 2. with different chromosome numbers within the same species; and 3. usefulness in developmental engineering [4, 11]. The present study will serve as a morphological report of a single time point in the developmental atlas of *A. speciosus.*

CONFLICT OF INTEREST. All authors declare no conflict of interest associated with this manuscript.

ACKNOWLEDGMENTS. This study was supported by Research and survey program of Akiyoshidai Karst Plateau Academic Center, Yamaguchi University to I.H. The authors thanks Laboratory of Veterinary Anatomy and Embryology, Yamaguchi University for participation in animal trapping.

REFERENCES

1. Abe, H. 2008. A Guide to the Mammals in Japan. 2nd Revised ed., Tokai University Press, Kanagawa.
2. Adams, F. W. and Hillemann, H. H. 1950. Morphogenesis of the vitelline and allantoic placentae of the golden hamster (*Cricetus auratus*). *Anat. Rec.* **108**: 363–383. [Medline] [CrossRef]
3. Ariyoshi, K., Miura, T., Kasai, K., Akifumi, N., Fujishima, Y. and Yoshida, M. A. 2018. Radiation-induced bystander effect in large Japanese field mouse (*Apodemus speciosus*) embryonic cells. *Radiat. Environ. Biophys.* **57**: 223–231. [Medline] [CrossRef]
4. Azuma, R., Hatanaka, Y., Shin, S. W., Murai, H., Miyashita, M., Anzai, M. and Matsumoto, K. 2020. Developmental competence of interspecies cloned embryos produced using cells from large Japanese field mice (Apodemus speciosus) and oocytes from laboratory mice (Mus musculus domesticus). *J. Reprod. Dev.* **66**: 255–263. [Medline] [CrossRef]
5. Carpenter, S. J. 1972. Light and electron microscopic observations on the morphogenesis of the chorioallantoic placenta of the golden hamster (*Cricetus auratus*). Days seven through nine of gestation. *Am. J. Anat.* **135**: 445–476. [Medline] [CrossRef]
6. Fukuyama, M. and Koike, K. 2007. Reproductive cycle of large Japanese field mouse *Apodems speciosus* in Gunma Insect World. Science Reports of the Faculty of Education. *Gunma University.* **56**: 61–68 (In Japanese).
7. Hanazaki, K., Tomozawa, M., Suzuki, Y., Kinoshita, G., Yamamoto, M., Irino, T. and Suzuki, H. 2017. Estimation of evolution rates of mitochondrial DNA in two Japanese field mouse species based on calibrations with quaternary environmental changes. *Zool. Sci.* **34**: 201–210. [Medline] [CrossRef]
8. Kaufman, M. H. 1995. The Atlas of Mouse Development, Rivised ed., Academic Press, London.
9. Kuwahara, S., Mizukami, T., Omura, M., Hagihara, M., Iinuma, Y., Shimizu, Y., Tamada, H., Tsukamoto, Y., Nishida, T. and Sasaki, F. 2000. Seasonal changes in the hypothalamo-pituitary-testes axis of the Japanese wood mouse (*Apodemus speciosus*). *Anat. Rec.* **260**: 366–372. [Medline] [CrossRef]
10. Matsunami, M., Endo, D., Saitou, N., Suzuki, H. and Onuma, M. 2017. Draft genome sequence of Japanese wood mouse, *Apodemus speciosus*. *Data Brief* **16**: 43–46. [Medline] [CrossRef]
11. Meguro, K., Komatsu, K., Ohdaira, T., Nakagata, N., Nakata, A., Fukumoto, M., Miura, T. and Yamashiro, H. 2019. Induction of superovulation using inhibin antiserum and competence of embryo development in wild large Japanese field mice (*Apodemus speciosus*). *Reprod. Domest. Anim.* **54**: 1637–1642. [Medline] [CrossRef]
12. Murakami, O. 1974. Growth and development of the Japanese wood mouse (*Apodemus speciosus*) I. The breeding season in the field. *Jap. J. Ecol.*

24: 194–206 (in Japanese, Abstract in English).
13. Okano, T., Onuma, M., Ishiniwa, H., Azuma, N., Tamaoki, M., Nakajima, N., Shindo, J. and Yokohata, Y. 2015. Classification of the spermatogenic cycle, seasonal changes of seminiferous tubule morphology and estimation of the breeding season of the large Japanese field mouse (*Apodemus speciosus*) in Toyama and Aomori prefectures, Japan. *J. Vet. Med. Sci.* **77**: 799–807. [Medline] [CrossRef]
14. Sakai, Y., Sakamoto, S.H., Kato, G.A., Iwamoto, N., Ozaki, R., Eto, T., Shinohara, A., Morita, T. and Koshimoto, C. 2013. Rearing method to induce natural mating of the large Japanese field mouse, *Apodemus speciosus*. *Honyurui kagaku (Mamm. Sci.)* **53**: 57–65 (in Japanese, Abstract in English).
15. Shimba, H. and Kobayashi, T. 1969. A Robertsonian type polymorphism of the chromosomes in the field mouse, *Apodemus speciosus*. *Jpn. J. Genet.* **44**: 117–122. [CrossRef]
16. Suzuki, Y., Tomozawa, M., Koizumi, Y., Tsuchiya, K. and Suzuki, H. 2015. Estimating the molecular evolutionary rates of mitochondrial genes referring to Quaternary ice age events with inferred population expansions and dispersals in Japanese Apodemus. *BMC Evol. Biol.* **15**: 187. [Medline] [CrossRef]
17. Theiler, K. 1989. The house mouse: Atlas of embryonic development, Springer-Verlag, New York.