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2 **Type:**

3 Note

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5 **Title:**

6 Histological analysis of implantation embryos in large Japanese field mouse (*Apodemus*
7 *speciosus*) and estimation of developmental stage.

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17 **Running head:**

18 IMPLANTATION EMBRYO OF *A. SPECIOSUS*

19

20 **Abstract:**

21 The large Japanese field mice (*Apodemus speciosus*) are small rodent specie endemic to
 22 Japan. The genetic characteristics of *A. speciosus* is different chromosome numbers
 23 within one species. Furthermore, *A. speciosus* is used for research in radiation and
 24 genetics. In this present study, a pregnant *A. speciosus* was obtained, and histochemical
 25 analysis of the implanted embryos was performed and compared with developmental
 26 stages of the mouse. Although there were some differences, the structures of the
 27 implanted embryos including the primitive streak and placenta of *A. speciosus* were
 28 similar to that of the mouse. Our study will be important report in the construction of a
 29 developmental atlas of *A. speciosus*.

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33 **Key words:**

34 *Apodemus speciosus*, Embryo, Histology, Implantation, Large Japanese field mouse

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Main Text:

The large Japanese field mice (*Apodemus speciosus*) is a small rodent that inhabits Japan and have diverged from its mouse and rat ancestor 1.5-4.4 hundred thousand years ago [3]. *A. speciosus*, found throughout the Japan except for Okinawa, is used as a model for studying geographic isolation of animals [11]. In addition, *A. speciosus* has distinctive genetic characteristics in that the number of chromosomes differs between western and eastern Japan. This karyotypic alternation in *A. speciosus* was caused by a Robertsonian translocation [10]. In recent years, *A. speciosus* has been used as animals to monitor the effects of radiation from nuclear power plant accidents [1].

During the genetic analysis of *A. speciosus*, which is our current project, A pregnant mice was obtained by chance. The morphology of the implantation sites was compared with Theiler's house mouse developmental staging [12] and summarized as a case report.

With the permission, *A. speciosus* were captured using the sharmans traps (LFA; Hoga, Kyoto, Japan). Each implantation site was identified (Fig. S1), fixed with Bouin solution and routinely embedded in paraffin. Serial section of 5µm were prepared using RM2245 (Leica, Wetzlar, German) and stained with H.&E.

The observation revealed typical primitive streak-like structures and two-three cavities except for R1 conception (Fig. 1). The primitive streak-like structures were similar to that of mouse embryos and consisted of three layers of cells, suggesting that the primitive streak structures containing these three cell layers were the primitive streak of *A. speciosus* (Fig. 1 Diagram; Pr). 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 placental region (Fig. 1 Diagram; Pl). Based on the orientation of primitive streak and placenta, the three cavities in the conception appeared to be ectoplacental cavity, exocoelom and amniotic cavity, respectively (Fig. 1 Diagram; Ec, Ex, A). Even in L1 preparation, where only two cavities were founded, part of partition between ectoplacental cavity and exocoelom could be observed. For R1 preparation, where only one cavity was founded, this might be a transverse section of primitive streak region, as there were three layers of cellular structures. Thus, these results indicated that the mice embryos used in this present study was 7.5-8.0 days old, which is corresponded to stage 11. However, there were some features that do not completely correspond the Theiler's developmental stages of mouse embryo; 1. allantois, which is stage-specific structure in exocoelom, could not be observed, 2. no clear blood islet could be observed. Therefore, *A. speciosus* embryos shows morphology basically similar to that of mouse development, but they also might differ slightly from mouse development.

As above, this present study showed the embryonic morphology of the stage 11 of *A. speciosus*. Although these embryonic ages were unknown, the gestation period of *A. speciosus* was 21-26 days [7], these embryos might be 7.9-10.4 days old. Methods of rearing and breeding the *A. speciosus* were being developed [9], and an atlas of embryonic development of *A. speciosus* could be created in the future. Furthermore, the whole genome sequence of *A. speciosus* had already been published [5], and *A. speciosus* can be established as a novel experimental rodent with the following characteristics; 1. rodents with seasonality in spermatogenesis and reproduction [4, 8], 2. with different

80 chromosome numbers within the same species, 3. pioneering developmental engineering
81 [2, 6]. This present study will be important as a morphological report of on point in the
82 developmental atlas of *A. speciosus*.
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Conflict of interest:

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

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140 **Figure Legends:**

141 **Figure 1**

142 Histological images of implantation sites in the *Apodemus speciosus*.

143 The implantation site number (L1-R2) and the preparation number of the serial sections

144 (#__) were shown in the upper left of images. The abbreviations in the diagram were as

145 follows; Pl: Placenta, Ec: Ectoplacental cavity, Ex: Exocoelom, A: Amniotic cavity and

146 Pr: Primitive streak. Bar; 100 μ m.

147

148 **Figure S1**

149 The implantation uterus of *Apodemus speciosus*.

150 The arrowheads indicated the implantation site number (L1-R2). Bar; 10 mm.

151

L1#21

L2#19

L3#15

L4#35

R1#35

R2#17

Diagram