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Basic reproductive features of captive Arabian spiny mice (*Acomys dimidiatus*)

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This study investigated the basic reproductive features of the Arabian spiny mouse (*Acomys dimidiatus*), a unique prosocial rodent. Two kinds of mating test revealed that the gestation period of *A. dimidiatus* is around 38 days, which is equivalent to that of its sibling species *A. cahirinus*, a widely used *Acomys* model species. Although it is considered difficult to estimate the gestation period of *Acomys* species because the species do not form typical copulatory plugs, we found that copulation events could be estimated by carefully observing the vulva. We also evaluated the reproductive performance of *A. dimidiatus* from past breeding records and found that the average litter size was 3.3 pups, similar to that of *A. cahirinus*. Furthermore, the weaning and mating success rates of *A. dimidiatus* were high (97.1% and 89.6%, respectively), indicating that this species is an excellent model for developmental and human gestation studies.

Keywords: *Acomys*; *Acomys dimidiatus*; gestation period; reproductive features

1. Introduction

African spiny mice (*Acomys*) are small rodents with prominent spiny hairs on their dorsal skin that inhabit the African region, the Levant, Mediterranean islands, and parts of Western Asia.[1] They are characterized by weak skin that is susceptible to splitting but which has greater regenerative ability compared to that of other mammals.[2–4] They also have a longer gestation period than laboratory mice and rats, and their pups are precocious and born at a more advanced stage of development.[5–7] These unique features are not found in laboratory mice or rats and make African spiny mice a precocious model organism for perinatal, regenerative, and embryonic development studies. To date, laboratory animal colonies of several *Acomys* species have been established, including *A. cahirinus* (Cairo spiny mice), which inhabits arid regions of Africa and Western Asia.[8]

A. dimidiatus (Arabian spiny mice), which inhabits Southwest Asia and Northeast Africa, is another Acomys species used in laboratories, and although the species is not as commonly used as *A. cahirinus*, breeding colonies are maintained in Japan and Switzerland.[9,10] Until the 2000s, there were conflicting hypotheses on the phylogenetic classification of *A. cahirinus* and *A. dimidiatus*, and no consensus on taxonomic boundaries or monophyly could be reached. *A. dimidiatus* and other related species were assigned to the *Acomys cahirinus–dimidiatus* complex because of their similarity in external appearance, molar morphology, and chromosome number, leading to the classification of *A. dimidiatus* as a subspecies of *A. cahirinus* without clear species boundaries.[11,12] Subsequent comparisons of mitochondrial DNA sequences and chromosomal banding patterns revealed that they are sibling species.[13–15] Although both species are thought to have similar biological features,

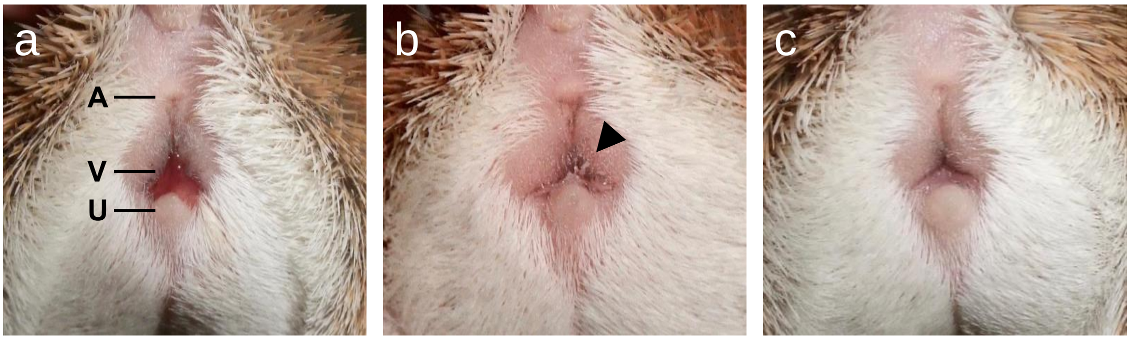

Figure 1. Appearance of the vulva of *Acomys dimidiatus*. a) Female after copulation in a mating experiment using postpartum estrus (1 day after mating), b) female with solidified white secretions adhering to the vaginal orifice, c) female with no mating experience. Arrowhead = solidified white secretions, V = vaginal orifice, A = anus, U = urethral orifice.

A. cahirinus is the leading *Acomys* model species for precocial development and regeneration studies, and no systematic characterization of *A. dimidiatus* has been conducted to date.

While maintaining a breeding strain derived from *A. dimidiatus*, we found out objects that looked like solidified white secretions attached to the vaginal orifice of females after copulation. In *A. cahirinus*, prominent semen has been observed in dissected reproductive tracts of females,[16] but it is difficult to observe vaginal plugs after copulation.[5,17] The solidified white secretion observed in *A. dimidiatus* may be the residue of a copulatory plug.

In this study, to demonstrate the potential of *A. dimidiatus* as a model organism for fetal development and pregnancy, we attempted to estimate the gestation period of spiny mice accurately both by postpartum mating and observation of the vaginal orifice after copulation using a laboratory strain of *A. dimidiatus*. Furthermore, we evaluated the delivered newborn pups and quantified the reproductive performance of animals in captivity to determine the basic reproductive features of this species.

2. Materials and Methods

Adults (2 to 12 months of age) of the *A. dimidiatus* Aco strain, which is maintained at the Department of Zoology at the Okayama University of Science, were used. The original Aco strain specimen was identified as *A. cahirinus* but a comparison of mitochondrial DNA sequences and chromosomal banding patterns revealed that it belonged to *A. dimidiatus*, and the strain has been maintained as an *A. dimidiatus* strain since then.[9] All animals were kept under photoperiods of 12 h light and 12 h darkness per day at 24ºC and were fed commercial pellets (Labo MR breeder; Nihon Nosan Kogyo. Co., Yokohama, Japan) and water *ad libitum*. All animal experiments were conducted following the Regulations for Animal Experiments of the Okayama University of Science. Our experimental protocols, including those involving animals (Exp2018–10), were approved by the Animal Experiments Committee of the Okayama University of Science.

2-1 Estimating gestation period via postpartum estrus (group 1)

A. cahirinus, like other rodents, has postpartum estrus.[18,19] Hence, we attempted to estimate the gestation period of *A. dimidiatus* via postpartum estrus. Ten pregnant females were kept alone until birth. Then, the pups were separated on the day of birth and each female was placed with a male. On the next morning, all males were separated, and the numbers of days until the females gave birth were recorded (the day of separation was set as day 1 of gestation).

2-2 Estimating gestation period via solidified secretions (group 2)

In all, 68 mating pairs were used to estimate the gestation period of *A. dimidiatus* via solidified secretions. On the evening of the day of mating, virgin females were placed in the same cage as a male. For each female, the vulva was observed visually every morning for up to 4 days. Females with a solidified white secretion at the vaginal orifice were immediately separated from males, and the number of days until delivery was recorded (the day of separation was set as day 1 of gestation).

2-3 Observation of the appearance of newborns

In all, 51 newborn pups from females of the above two groups were used to observe the appearance of *A. dimidiatus* newborns. After euthanasia by isoflurane overexposure, body weight and head–body length were recorded, as well as the presence or absence of finger deviations, incisor tooth eruption, eyelid and pinna opening, beard, and body hair.

2-4 Estimation of productive mating frequency, litter size, and weaning rate

Data on mating success, litter size, and weaning rate were collected from maintenance records of the *A. dimidiatus* Aco strain from 2016 to 2020, which was maintained by random mating. The data included 148 natural mating pairs. Every delivered litter was considered a successful mating, and mating success was calculated for 128 pairs that started mating between 1 and 3 months of age. Of the pairs that did not achieve pregnancy, those that had been housed together for a short period (less than 90 days) were excluded from this study.

2-5 Statistical Analysis

The measurements obtained from the observation were statistically analyzed using the statistical Rcmdr package version 2.7-1[20,21] with R version 4.0.4.[22]

3. Results

3-1 Gestation period

Mating experiments showed that the mean gestation period in females who gave birth was 38.2 ± 0.4 days in group 1 based on postpartum estrus and 38.3 ± 0.7 days in group 2 based on solidified secretions. There were no differences in gestation period between the two groups, with 38 days being the most predominant period (combined 38.2 ± 0.6 days) (Table 1).

In group 1, no apparent copulatory plug or secretion was observed at the vaginal orifice of females on the day after mating, but hemorrhage was observed in 7 out of 10 mated females, presumably due to copulation (Fig. 1a), and 6 of these females gave birth. In group 2, no apparent copulatory plug was observed either. However, 23 out of 68 females had solidified white secretions (Fig. 1b), and 7 of these females gave birth. No secretions were observed in the remaining 45 females, but 4 of them gave birth.

Table 1. Gestation period of *Acomys dimidiatus.*

Group*	n	Gestation period	
		Mean ± SD	Range
Group 1 (postpartum estrus)	6	38.2 ± 0.4	38–39
Group 2 (solidified secretions)	7	38.3 ± 0.7	37–39

*Gestation periods were estimated by using postpartum estrus (Group 1) and by confirming of solidified white secretions (Group 2) (see materials and methods).

3-2 Development of newborns

Observations of the external developmental status of litters from both groups on the day of birth showed divergence of fingers, eruption of incisors, opening of eyelids and auricles, whiskers, and fur in all 51 newborns. The body weight and external measurements of the newborns were summarized by gestation period (Table 2), although the values for newborns that had a 37-day gestation period may have been inaccurate due to the small number of newborns. Overall, the later the birth, the more advanced the development of the newborns. There was a statistically significant difference in body weight, head and body length, tail length, ear length, and hindfoot length between the newborns of the 38- and 39-day gestation periods ($p < 0.05$, one-way ANOVA followed by Tukey-Kramer test).

3-3 Basic reproductive performance of the captive *A. dimidiatus* strain based on breeding records

The breeding data collected from maintenance records of the *A. dimidiatus* Aco strain from 2016 to 2020 showed that 754 pups were born in 232 litters over 5 years, with a mean litter size of 3.3 ± 1.4 (range 1 to 9). The weaning rate was 97.1% (608 out of 628

Table 2. Body weight and external measurements in newborns of *Acomys dimidiatus* for each gestation period.

Gestation period	n	Body weight (g)		Head-body length (mm)		Tail length (mm)		Ear length (mm)		Hind foot length (mm)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
37 days	3	5.4 ± 0.2	5.2–5.6	49.6 ± 0.5	48.9–50.0	35.6 ± 0.2	35.4–35.8	9.8 ± 0.6	9.0–10.3	15.9 ± 0.4	15.5–16.4
38 days	36	5.3 ± 0.4	4.2–5.9	49.2 ± 1.8	44.7–52.2	35.3 ± 1.4	31.5–37.5	9.8 ± 0.4	8.9–10.9	15.9 ± 0.4	14.9–16.7
39 days	12	6.0 ± 0.3	5.4–6.5	51.4 ± 2.3	46.7–55.0	37.9 ± 1.2	36.0–39.8	10.2 ± 0.5	9.3–11.0	16.5 ± 0.4	15.6–17.1

pups were weaned), and the mating success was 89.6% (120 out of 128 mating pairs gave birth).

4. Discussion

Our results indicate that the gestation period of captive *A. dimidiatus* is on average 38 days (37–39 days), equivalent to the period reported for *A. cahirinus*;[18,23-27] this is considered a common feature of *Acomys* species. In addition, most females were pregnant in the mating experiment using postpartum estrus, implying that *A. dimidiatus* exhibits postpartum estrus, as does *A. cahirinus*. Furthermore, the gestation period estimated via postpartum estrus was similar to that estimated by observation of the vaginal orifice (presence of solidified secretions), implying that the phenomenon of delayed implantation observed in many mammal species does not occur in *A. dimidiatus*. Although a difference in the gestation period of ± 1 day (37–39 days) was observed, the degree of growth at birth was different in each case, and the fact that the newborns grew more progressively with a longer gestation period indicates that growth in the fetal period was uniform. In a previous study, copulatory plugs were not formed in spiny mice,[23] but we observed solidified white secretions around the vaginal orifice of females. The male ejaculates of *A. cahirinus* may not coagulate in this species, which may explain why plugs do not form.[28] In the present study, we did not observe any typical plugging of the vagina. However, about 30% of the females with secretions at the vagina gave birth, and conversely, most of the animals for which no solid material was observed did not give birth, implying that this material may have been the residue of a semen plug or similar material produced by copulation. Although it may not be possible to confidently determine successful pregnancy by observing secretions adhering to the vaginal orifice, it may allow us to determine the date of pregnancy in spiny mice without using postpartum estrus.

The average litter size of *A. cahirinus* is around two to three (ranging from one to four).[18,23-27] The mean litter size of *A. dimidiatus* calculated from the breeding colony records was approximately three, which is equivalent to that of *A. cahirinus*. Furthermore, the weaning rate was very high at 97%, which is typical for a K-strategist. There are records of births of more than four pups per litter (up to nine pups) in this strain, so it seems that spiny mice do occasionally give birth to a higher number of pups.

This study provides insight into the reproductive features of *A. dimidiatus*. *A. cahirinus* has a long ovulatory cycle of 11 days[28] and subsequent menstruation,[29] which are very rare in rodent species. The cycle of ovulation and the presence of menstruation in *A. dimidiatus* are unconfirmed. To demonstrate the potential of *A. dimidiatus* as a model organism for human gestation and fetal development, and to enable comparative biological analysis using sister species, further investigation of the reproductive features of *A. dimidiatus* is necessary.

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