Asian Journal of Research in Zoology

1(1): 1-7, 2018; Article no.AJRIZ.43385

Formation and Diachronic Changes of Placental Scars in the House Shrew (*Suncus murinus*)

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KN and KM conceived and designed the experiments. Both of them performed the experiments, analysed the data and wrote the paper. Author KM revised and finalised the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIZ/2018/v1i11100
Editor(s):
(1) Dr. P. Murali, Assistant Professor, Tamil Nadu veterinary & Animal Sciences University, India.
Reviewers:
(1) Hakan Nazik, Health Sciences University, Turkey.
(2) Alok Nahata, Malaysia.
(3) Pall Emoke, University of Agricultural Sciences and Veterinary Medicine, Romania.
Complete Peer review History: http://www.sciencedomain.org/review-history/26293

Short Research Article

Received 21st May 2018
Accepted 4th August 2018
Published 19th September 2018

ABSTRACT

In the present study, diachronic changes in fresh and stained placental scars of postpartum house shrews *(Suncus murinus*) were studied and compared with the actual number of offspring. In non-stained fresh uteri, the placental scars were barely visible externally, except in some exceptional cases. In stained uteri, placental scars were observed in all specimens, even in the oldest animals evaluated (12 months postpartum). The study estimated the number of offspring and time of parturition using the degree of staining in the scars and the change in their forms. As the time postpartum increased, the stained placental scars became discoloured and shrank, making it difficult to distinguish between the scars from normal parturition and those that formed following halted development after implantation. Placental scars provided an invaluable method for estimating the female reproductive state in wild mammals. In captive house shrews, however, placental scars were barely detected in non- stained fresh uteri. Moreover, placental scars were observed in older animals at 12 months postpartum. It can be concluded that, it is not feasible to estimate the number of offspring or time of parturition based on placental scars in wild shrews, which are believed to undergo multiple parturitions a year.

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Keywords: Suncus murinus; house shrew; placental scar; litter size; uterus.

1. INTRODUCTION

The placental scar is the rudiment that forms when the placenta separates from the uterine tissues. The scar becomes pigmented due to phagocytosis by macrophages of remaining placenta and blood [1]. The number of placental scars should equal the number of post- implantation embryos [2,3]. As time passes after parturition, the colour and shape of the placental scar changes and these changes may be useful for estimating the time of parturition [4,5]. Consequently, placental scars have been used to estimate the number of litters and parturition history in various mammalian species [*e.g.* 1,6,7,8,9]. However, the degree of change in placental scars with the passage of time postpartum varies across species [10]. Therefore, evaluation references suitable for each species are necessary.

The house shrew (*Suncus murinus*) (Soricidae, Soricomorpha) is a small mammal found in the tropics and subtropics (South and Southeast Asia) [11,12], where it is regarded as a pest because it inhabits human residences and feeds on human food and garbage [13]. It is also a potential host and transmitter of pathogenic bacteria causing contagious diseases in humans [14,15,16]. Zoonoses are a particular concern in densely populated urban areas. Therefore, an understanding of this mammal's reproductive conditions would provide invaluable information for taking effective counter measures against such infectious diseases [17]. To our knowledge, no study has examined placental scars in this species till date. Therefore, we examined the placental scars in the house shrew for assessing its population dynamics through female reproductive success. We observed diachronic changes in the placental scars of captive house shrews and evaluated the changes in the colour and shape of these scars with respect to the actual litter size.

2. MATERIALS AND METHODS

2.1 Animals

The KAT laboratory suncus strain was used; this strain was derived from wild populations in Kathmandu, Nepal [18]. Virgin adult females were mated with males overnight. In consideration of phenotypic individual differences, the matings were conducted so that multiple parous females can be secured at each stage. Finally, five females were sampled each month from 1 to 6 months and again at 8 months after parturition and three and two females were sampled 10 and 12 months after parturition, respectively. The number of young born in each litter and the date of birth were recorded within 3 days after parturition. The animals were housed individually in plastic cages containing wood chips under a 12L:12D photoperiod at 25℃. Water and food (commercial trout pellets; Feedone Co., Ltd., Yokohama, Japan) were available *ad libitum*. All animal experiments were conducted in accordance with the Regulations for Animal Experiments of the Okayama University of Science. The experimental protocols, including those involving animals (Exp2017-06), were approved by the Animal Experiments Committee of the University.

2.2 Preparation of the Reproductive Tracts

Isolated uterine horns were first examined under a stereomicroscope (SZX-ILLB100; Olympus Corporation, Tokyo, Japan) to determine the number of placental scars prior to staining the tissues. The staining method was based on the Berlin blue (Perls' Prussian blue) reaction [19] previously used on laboratory rats [20]. The uteri were fixed in 4% paraformaldehyde for overnight, dehydrated in a graded ethanol series, and stained with a freshly prepared acid ferrocyanide solution (equal parts 2% aqueous potassium ferrocyanide and 2% aqueous hydrochloric acid) for 30 minutes. The samples were washed thoroughly in distilled water. After staining, the uterine horns were opened longitudinally and examined under a stereomicroscope. The placental scars were first categorised according to the intensity of pigmentation to differentiate full-term from prematurely terminated pregnancies [5,10,21]. Briefly, placental scars from full-term pregnancies formed two dark parallel bands across the uterus with dense pigmentation in the tissue between the two bands. However, the placental scars resulting from prenatal mortality were paler and composed of one or two thin dark lines with little or no pigmentation between them.

3. RESULTS

3.1 Microscopic Observations of Placental Scars before Staining

External observations of unstained fresh uteri from the house shrews (n=40) at the different

times postpartum, failed to detect any placental scars except in one animal at 4 months postpartum (animal ID 503a) (Fig. 1). Transillumination of the uterus with a strong light revealed thin shadowy images suggestive of placental scars in some samples (data not shown), although these were not confirmed and were recorded as “no placental scar confirmed”.

3.2 Microscopic Observation of Placental Scars after Staining

In the uteri stained with Berlin blue, most placental scars were derived from the full-term pregnancies. Such placental scars, observed in all animals (n=40) at 1–12 months postpartum, were entirely stained blue, with two dense blue peripheral bands and a paler central crater (Fig. 2). We also found some scars that likely indicated ceased development post-implantation. These exhibited a paler stain and smaller size than those inferred to result from full-term pregnancies.

As the time postpartum progressed, the placental scars became paler and smaller (Fig. 3), although the densely coloured peripheral bands were still observed at 12 months postpartum. The central crater remained densely stained from 1 to 3 months postpartum but became paler and smaller after 4 months postpartum. The placental scars after 5 months postpartum were easily distinguishable from those at 1–3 months postpartum. No great change after 5 months postpartum was observed. The placental scars were round and large at 1-3 months postpartum but narrowed gradually from the mesometrium side toward the anti-mesometrium after 4 months postpartum.

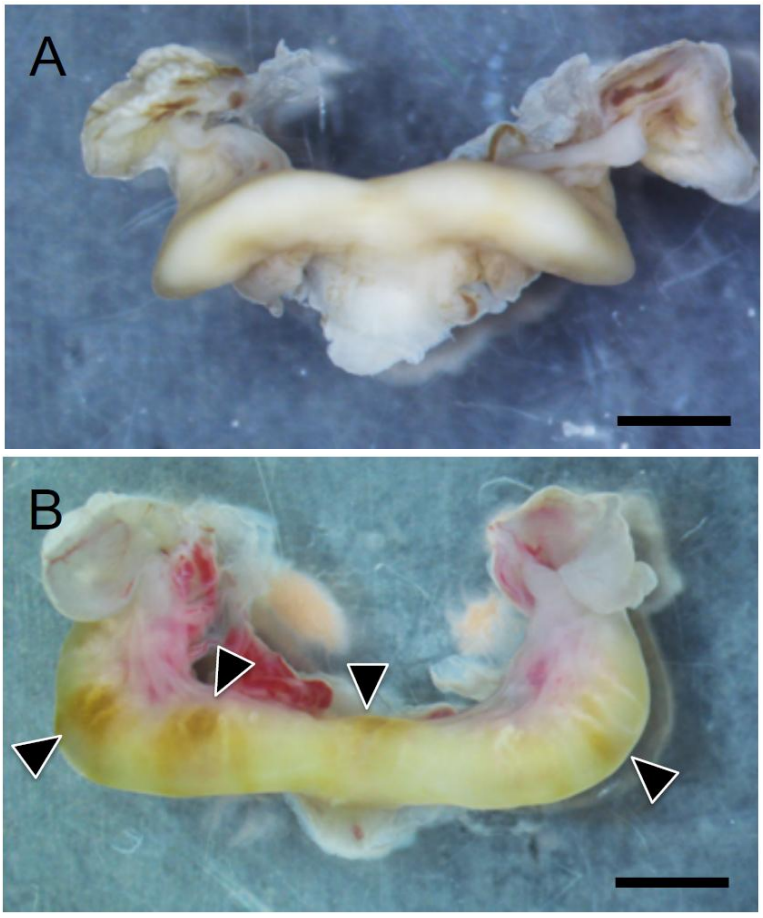

Fig. 1. Untreated uteri of house shrews.(A) No placental scars were detected in most animals at different times postpartum (5 months postpartum is shown as an example here), (B) with the exception of placental scars (arrowheads) identified in one animal at 4 months postpartum. Scale bar, 2 mm

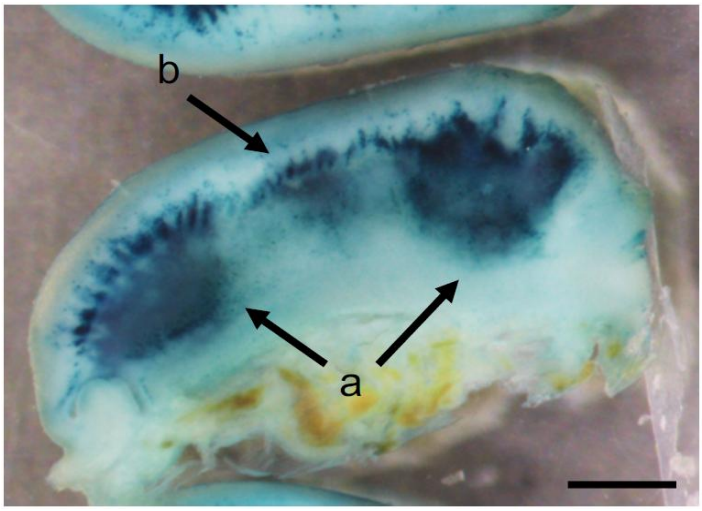

Fig. 2. Longitudinal section of the uterus of a house shrew (1 month postpartum is shown as an example here). Placental scars indicating (a) full-term pregnancies and (b) prenatal mortalities. Scale bar, 1 mm

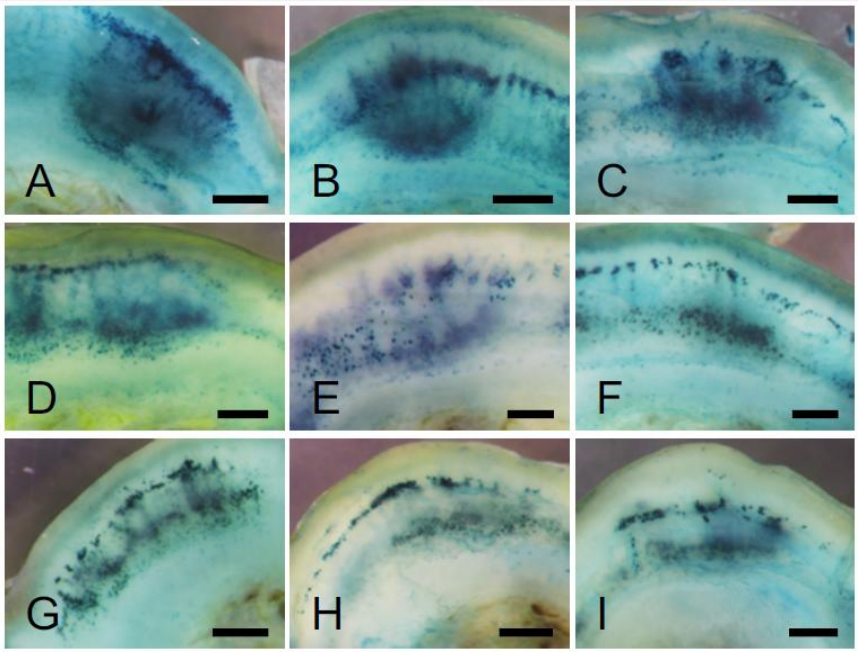

Fig. 3. Change in placental scars with time since parturition in house shrews, evaluated at (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, (F) 6, (G) 8, (H) 10, and (I) 12 months postpartum. Scale bar, 0.5 mm

3.3 Comparison between Placental Scar Count and Recorded Litter Size

The number of placental scars obtained from full-term pregnancies and the recorded litter size for the 40 sampled animals were compared. The mean ± standard deviation (SD) placental scar count per litter was 4.78 ± 1.33 and the mean ± SD litter size was 4.45 ± 1.45. The actual number of offspring never exceeded the number of observed placental scars, while the number of placental scars that resulted from full-term pregnancies exceeded the actual number of offspring in 11 animals (27.5%). A greater proportion of such animals were recorded at later times after parturition (Table 1).

4. DISCUSSION

4.1 Identification of Placental Scars from Untreated and Stained Uteri

In most of the examined house shrews, observations of fresh uteri at autopsy failed to reveal placental scars (Fig. 1). Unlike the house shrew, placental scars are detectable in untreated fresh uteri of rodents, such as laboratory mice and rats [22]. Placental scars were identified in one animal, and transmitted light showed shadows likely representing placental scars in some samples. Therefore, incision of the uterus at autopsy may reveal placental scars, although it is not understood why they were detected only in one animal. In future, the sample size should be greater to confirm the observability of placental scars without staining.

By contrast, the house shrew uteri stained with Berlin blue revealed placental scars at all stages postpartum. Differences in stain colour and shape were observed over time (Fig. 3). These differences in the degree of staining may be useful for estimating the number of offspring and parturition time.

4.2 Reliability of Estimation of House Shrew Reproductive Conditions Using Placental Scars

The number of placental scars exceeded the actual number of offspring in 27.5% of the animals. More than half of these animals were over 5 months postpartum (Table 1). After 5 months, the placental scars were paler and smaller (Fig. 3). This makes it difficult to distinguish placental scars remaining postpartum and to estimate the number of offspring correctly with time. Infanticide and cannibalism shortly after parturition and miscarriage (stillbirth) during the perinatal period are the likely causes of the greater number of placental scars postpartum relative to the actual number of offspring [8, 21, 23]. However, it is impossible to exclude the possibility that a placental scar associated with no parturition was included as a normal placental scar resulting from a full-term pregnancy.

Placental scars in one animal at 12 months after the last parturition were also observed. In wild house shrews, in which multiple parturitions are expected, overlapping placental scars make it difficult to estimate the correct number of offspring and times of parturition. The average longevity of wild soricids is 2-17 months [24]. Species in the genera *Suncus* and *Crocidura* inhabiting temperate or tropical regions may breed year-round [24, 25, 26], and some animals may have multiple parturitions in a year. It is unknown whether the diachronic findings of placental scars in the house shrew are common to other species of genus *Suncus* or related species. However, when estimating the number of offspring and parturition times using placental scars in wild house shrews, the reliability is likely to be low unless these issues are disregarded.

Table 1. Litter size, uterus wet weight and placental scar counts (mean ± SD) in house shrews

Months postpartum	n	Litter size	Uterus weight (g)	Placental scars from full-term pregnancies	All placental scars	% of animals*
1	5	4.20±1.48	0.11±0.02	4.40±1.67	5.20±1.64	20% (1/5)
2	5	4.20±2.59	0.07±0.02	4.40±2.30	5.60±1.67	20% (1/5)
3	5	4.00±1.41	0.06±0.02	4.20±1.10	4.80±0.45	20% (1/5)
4	5	4.80±1.30	0.08±0.03	5.00±1.22	6.60±0.89	20% (1/5)
5	5	4.80±1.48	0.07±0.05	5.40±1.52	6.40±0.89	40% (2/5)
6	5	4.80±1.79	0.05±0.01	5.20±1.30	5.80±1.30	40% (2/5)
8	5	4.60±0.55	0.05±0.01	5.00±0.00	6.20±1.30	40% (2/5)
10	3	4.33±1.53	0.05±0.01	5.00±1.00	5.33±0.58	33.3% (1/3)
12	2	4.00±0.00	0.04±0.01	4.00±0.00	5.00±0.00	0% (0/2)
Total	40	4.45±1.45	0.07±0.03	4.78±1.33	5.72±1.22	27.5% (11/40)

** Percentage of animals whose placental scar counts from full-term pregnancies exceeded the litter size*

5. CONCLUSIONS

In untreated fresh uteri, no placental scars were observed in the majority of the evaluated house shrews. After staining with Berlin blue, placental scars were observed in all animals from 1 to 12 months postpartum. It is possible to estimate the number of offsprings and time of delivery according to the difference in the degree of staining, but it may be difficult to discriminate normal placental scars from full-term pregnancies with time. Placental scars were also observed in animals at 12 months postpartum, so caution is required when applying this method to house shrews, which breed annually.

ACKNOWLEDGEMENTS

We appreciate English proofing for earlier versions of the manuscript by Prof. H. Takasaki (OUS Department of Zoology).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ruette S, Albaret M. Reproduction of the red fox *Vulpes vulpes* in western France: does staining improve estimation of litter size from placental scar counts? Eur J Wildl Res. 2011;57:555–564.
2. Stewart I, Peel S. Granulated metrial gland cells at implantation sites of the pregnant mouse uterus. Anat Embryol (Berl). 1980; 160:227-238.
3. Jbara K, Stewart I. Granulated metrial gland cells in the uterus and labyrinthine placenta of inbred and outbred pregnancies in mice. J Anat. 1982; 135:311-317.
4. Wydoski RS, Davis DE. The occurrence of placental scars in mammals. Proc Pa Acad Sci. 1961;35:197-204.
5. Martin KH, Stehn RA, Richmond ME. Reliability of placental scar counts in the prairie vole. J Wildl Manage. 1976;40:264-271.
6. Helle E, Kauhala K. Reproduction in the Raccoon Dog in Finland. J Mamm. 1995; 76:1036-1046.
7. Mowat G, Boutin S, Slough BG. Using placental scar counts to estimate litter size and pregnancy rate in lynx. J Wildl Manage. 1996;60:430-440.
8. Bray Y, Marboutin E, Péroux R, Ferron J. Reliability of stained placental-scar counts in European hares. Wildl Soc Bull. 2003; 31:237-246.
9. Santicchia F, Romeo C, Grilli G, Vezzoso S, Wautersa LA, Mazzamuto MV, Martinoli A, Ferrari N. The use of uterine scars to explore fecundity levels in invasive alien tree squirrels. Hystrix It J Mamm. 2015; 26:95-101.
10. Elmeros M, Hammershøj M. Experimental evaluation of the reliability of placental scar counts in American mink (*Mustela vison*). Eur J Wildl Res. 2006;52:132-135.
11. Nowak RM. Walker's mammals of the world, Sixth edition, Volume II. John Hopkins University Press: Baltimore; 1999.
12. Wilson DE, Reeder DM. Mammal species of the world. 3rd ed. The Johns Hopkins University Press: Baltimore; 2005.
13. Liat LB. The house rodents and house shrew in Malaysia and Southeast Asia. UTAR Agr Sci J. 2015;1**:**43-50.
14. Marshall JD, Quy DV, Gibson FL, Dung TC, Cavanaugh DC. Ecology of plague in Vietnam. I. Role of *Suncus murinus*. Proc Soc Exp Biol Med. 1967;124:1083-6.
15. Rahelinirina S, Rajerison M, Telfer S, Savin C, Carniel E, Duplantier JM. The Asian house shrew *Suncus murinus* as a reservoir and source of human outbreaks of plague in Madagascar. PLoS Negl Trop Dis. 2017;11:e0006072.
DOI: 10.1371/journal.pntd.0006072
16. Wang B, Cai CL, Li B, Zhang W, Zhu Y, Chen WH, Zhuo F, Shi ZL, Yang XL. Detection and characterization of three zoonotic viruses in wild rodents and shrews from Shenzhen city, China. Virol Sin. 2017;32:290-297.
DOI: 10.1007/s12250-017-3973-z
17. Takakura KI, Abe T, Doi K, Sanada SI, Hase A. Bayesian estimation of population dynamics of feral cats in Osaka City, Japan. Jpn J Environ Entomol Zool. 2013; 24:133-141. Japanese.
18. Oda SI, Koyasu K, Shrestha KC. Breeding of the house musk shrew, *Suncus murinus* originating from a wild population in Kathomandu, Nepal. Annu Rep Res Inst Environ Med, Nagoya Univ. 1992;43:239-240. Japanese.
19. Nayak R. Histopathology techniques and its management. Jaypee Brothers Medical Publishers: London; 2017.
20. Yamada T, Hara M, Ohba Y, Inoue T, Ohno H. Studies on implantation traces in

Rats I. Size, observation period and staining. Jikken Dobutsu. 1985;34:17-22. Japanese.
21. Strand O, Skogland T, Kvam T. Placental scars and estimation of litter size: An experimental test in the Arctic fox. J Mamm. 1995;76:1220-1225.
22. Aplin KP, Brown PR, Jacob J, Krebs CJ, Singleton GR. Field methods for rodent studies in Asia and the Indo-Pacific. Australian Centre for International Agricultural Research: Canberra; 2003.
23. Elmeros M, Pedersen V, Wincentz TL. Placental scar counts and litter size estimations in ranched red foxes (*Vulpes vulpes*). Mammal Biol. 2003;68:391-393.
24. Churchfield S. The natural history of shrews. A & C Black Ltd.: London; 1990.
25. Chang CH, Lin JY, Lin LK, Yu JYL. Annual reproductive patterns of male house shrew, *Suncus murinus*, in Taiwan. J Mamm. 1999;80:845-854.
26. Chang CH, Lin JY, Lin LK, Yu JYL. Annual reproductive patterns of female house shrew, *Suncus murinus*, in Taiwan. Zool Sci. 1999;16:819-826.

Peer-review history:
The peer review history for this paper can be accessed here:
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