NOTE
Theriogenology

Accuracy of follicle count and ovulation confirmation using magnetic resonance imaging in microminipigs with normal estrus cycles

Masaki TAKASU1,2), Ryoko BABA1), Satsuki OWADA1), Kotono NAKAMURA1), Julio ALMUNIA1), Noriko NISHII1) and Hitoshi KITAGAWA1)

1) Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
2) Education Center for Food Animal Health (GeFAH), Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

ABSTRACT.
Magnetic resonance imaging (MRI) is suggested to be useful for counting follicles and confirming ovulation in microminipigs. However, its accuracy is unknown. We have compared the number of follicles counted by MRI to that of corpus hemorrhagicum confirmed directly by visual inspection. The follicles of 17 microminipigs were counted by using ovarian MRI on a 0.4 Tesla MRI System every 24 hr after estrus until follicle images disappeared. Then, we performed laparotomy to count their corpus hemorrhagicum. Significant correlation was observed between follicle counts obtained using MRI (5.18 ± 1.78 per head) and the numbers of corpus hemorrhagicum (5.47 ± 1.74 per head). In conclusion, follicle counts using 0.4-T MRI were reliable, and confirmed microminipig ovulation.

KEY WORDS: follicle count, magnetic resonance imaging, Microminipigs, ovulation confirmation

Microminipigs are extremely small miniature pigs whose body size is about half that of other miniature pigs, and approximately 1/10th that of domestic pigs. They weigh approximately 10 kg at nine months of age and 20–30 kg in adulthood [1, 2]. Pigs are good candidate animals for translational research due to their physiological and anatomical similarities to humans [3, 4, 8]. However, domestic pigs and other miniature pig species are sometimes too large to use for research. In contrast, microminipigs have an advantage as experimental animals because of their small size.

Ovarian imaging is very useful for functional and morphological evaluation and for gaining an understanding of the animal in the field of reproductive research. Magnetic resonance imaging (MRI) can be used to observe ovarian activity and to confirm ovulation in microminipigs [5]. However, ovulation was only assumed to have finished when existing follicle images disappear [5], and therefore the accuracy of follicle counts and ovulation confirmation using MRI was not clear. Here, we directly confirmed the presence of corpus hemorrhagicum and counted them by laparotomy following ovulation confirmation by MRI, and then evaluated the accuracy of the follicle count and ovulation confirmation using MRI.

Seventeen microminipigs aged 22–27 months and weighing 20.6 ± 3.8 kg (mean ± standard deviation) at the time of the experiment were used in this study. The microminipigs were purchased from Fuji Micra Inc. (Fujinomiya; Shizuoka, Japan). They were kept in a controlled room at 24°C (21–27°C) and a humidity of 70–80%. The room was maintained at a 12-hr light/12-hr dark lighting cycle, starting at 0600. The microminipigs were fed once daily with Herb Kodakara 74 (Marubeni Nisshin Feed Co., Ltd.; Tokyo, Japan) according to the manufacturers’ recommendations. The animals had free access to water. This study was approved by the Committee for Animal Research and Welfare of Gifu University (#15084).

The estrus cycle was confirmed based on behavioral changes, vulval swelling, and back-pressure testing [6]. Immediately after estrus confirmation, MRIs were acquired at a 24-hr interval until all the existing follicle images disappeared [5]. Ovulation was assumed to have finished within 24 hr of the day that all follicle images disappeared. For MRI acquisition, the pigs were sedated by intramuscular administration of 0.015 mg/kg medetomidine, 0.15 mg/kg midazolam, and 0.12 mg/kg butorphanol (MMB) [7]. MRI acquisition over the area within a range of 180 mm from the hindmost teat was performed using a 0.4-T MRI System (Hitachi Medical Corporation; Chiba, Japan). Pelvic multi-slice T2-weighted spin-echo image acquisition was performed in the sagittal and transaxial planes using a respiratory gating system (repetition time/echo time, 12,000/104 msec; flip angle, 90°; field of view, 180 mm; slice thickness, 3 mm) (Fig. 1).

Received: 28 July 2017
Accepted: 31 October 2017
Published online in J-STAGE: 28 November 2017

J. Vet. Med. Sci.
80(1): 125–127, 2018
doi: 10.1292/jvms.17-0417

©2018 The Japanese Society of Veterinary Science
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)
Laparotomy was done under anesthesia using isoflurane immediately after ovulation was confirmed by MRI. Pigs were sedated by intramuscular administration of MMB. Following sedation, flunixin meglumine (1 mg/kg) was intravenously administered through the ear blood vessels for pain control. Then, anesthesia was administered using an anesthetic mask. A surgical incision of approximately 5 cm was made into the abdominal wall between the hindmost teats, and the number of corpus hemorrhagicum on the bilateral ovaries were visually counted (Fig. 2). After the investigation, the animals were recovered and robenacoxib tablets (1/2 tablet per day) were administrated for three days as an anodyne.

Using image-processing software dedicated to DICOM images (OsiriX, v. 4.1.2, Pixmeo Sàrl, Bernex, Switzerland), the number of ovarian follicles were counted by two examiners using sagittal and transaxial images. Pearson’s correlation coefficient between the follicle counts and the number of corpus hemorrhagicum was calculated to determine the accuracy of follicle observation using MRI.

Fig. 1. Abdominal magnetic resonance images of a microminipig for ovarian observation in the sagittal (A) and transaxial (B) planes. Follicles are confirmed as hyper-intense structures (arrowhead).

Fig. 2. Corpus hemorrhagicum (arrowheads) were visually counted by laparotomy.

Fig. 3. Correlation coefficient (r) for the number of follicles observed by magnetic resonance imaging and the number of corpus hemorrhagicum (r=0.782, P<0.01). R²=coefficient of determination.
Corpus hemorrhagicum were confirmed in all the microminipigs at laparotomy. The average number of follicles counted using MRI was 5.18 ± 1.78 per head, and the average corpus hemorrhagicum count was 5.47 ± 1.74 per head. A significant correlation was observed between the follicle counts using MRI and the number of corpus hemorrhagicum \((n=17, r=0.782, P<0.001)\) (Fig. 3).

Follicle counts and the number of corpus hemorrhagicum were correlated but did not match perfectly. This could be explained by the disappearance of some follicles without ovulation or by follicles that were not detected under the MRI conditions used. Moreover, there may be some limitations in distinguishing adjoining corpus hemorrhagicum. However, these results support the use of MRI for counting follicles in microminipigs for research and clinical purposes. Moreover, ovulation can be confirmed by the disappearance of follicle images on MRI.

MRI is useful when confirming the presence or absence of follicles, even with use of a 0.4-T MRI device. Ovarian follicles were relatively easy to observe using MRI because no hyper-intense circular structure with a size of 10 mm or less was observed within the abdominal cavity, other than the ovarian follicles [5]. Additionally, unlike in ultrasonography, in which probe-operating techniques affect the results, follicle confirmation by MRI did not depend upon the examiner’s technique.

In this study in which a 0.4-T MRI scanner was used, the boundaries of the follicle images were blurred and became unclear as the number of follicles increased. Therefore, the 0.4-T MRI may have limitations for ovarian observation under abnormal conditions, such as when ovaries are treated using hormones for superovulation, which could lead to the presence of many follicles. However, the accuracy of follicle count and other ovarian observations may increase with the use of high-resolution MRI. In addition to using higher-resolution MRI, we may also be able to increase the accuracy of follicle counts by obtaining coronal plane images.

ACKNOWLEDGMENT. We would like to thank Dr. Okada, M. for her insightful comments and suggestions.

REFERENCES

1. Kaneko, N., Itoh, K., Sugiyama, A. and Izumi, Y. 2011. Microminipig, a non-rodent experimental animal optimized for life science research: preface. *J. Pharmacol. Sci.* **115**: 112–114. [Medline] [CrossRef]

2. Kawaguchi, H., Yamada, T., Miura, N., Takahashi, Y., Yoshikawa, T., Izumi, H., Kawarasaki, T., Miyoshi, N. and Tanimoto, A. 2012. Reference values of hematological and biochemical parameters for the world smallest microminipigs. *J. Vet. Med. Sci.* **74**: 933–936. [Medline] [CrossRef]

3. Klymiuk, N., Seeliger, F., Bohlooly-Y, M., Blutke, A., Rudmann, D. G. and Wolf, E. 2016. Tailored pig models for preclinical efficacy and safety testing of targeted therapies. *Toxicol. Pathol.* **44**: 346–357. [Medline] [CrossRef]

4. Köhn, F. 2012. History and development of miniature, micro-, and minipigs. pp. 3–15. In: The Minipig in Biomedical Research (McAnulty, P.A., Dayan, A.D., Ganderup, N., Hastings, K. L. eds.), CRC Press, Boca Raton.

5. Maeda, M., Takashima, S., Takasu, M., Mori, N., Matsubara, T., Almunia, J., Imaeda, N., Ando, A. and Kitagawa, H. 2016. Magnetic resonance imaging of ovarian activity in microminipigs showing normal estrous cycles. *In Vivo* **30**: 35–40. [Medline]

6. Noguchi, M., Miura, N., Ando, T., Kubota, C., Hobo, S., Kawaguchi, H. and Tanimoto, A. 2015. Profiles of reproductive hormone in the microminipig during the normal estrous cycle. *In Vivo* **29**: 17–22. [Medline]

7. Takasu, M., Tsuji, E., Imaeda, N., Matsubara, T., Maeda, M., Ito, Y., Shibata, S., Ando, A., Nishii, N., Yamazoe, K. and Kitagawa, H. 2015. Body and major organ sizes of young mature microminipigs determined by computed tomography. *Lab. Anim.* **49**: 65–70. [Medline] [CrossRef]

8. Vodicka, P., Smetana, K. Jr., Dvoránková, B., Emerick, T., Xu, Y. Z., Ourendik, J., Ourendík, V. and Motlík, J. 2005. The miniature pig as an animal model in biomedical research. *Ann. N. Y. Acad. Sci.* **1049**: 161–171. [Medline] [CrossRef]