

## TECHNICAL NOTE

# Immobilization Technique for High-Resolution MR Imaging of the Testes

Masayuki Yamaguchi\* and Hirofumi Fujii

Techniques for testis immobilization can facilitate high-resolution MR imaging applications for testicular diseases by assuring good positioning of the testis on small radiofrequency coils and reducing motion artifacts. We tested negative pressure suction to immobilize the testis of rats during MR image acquisitions. Suction pressure between  $-5$  and  $-10$  kPa assured good positioning, suppressed motion artifacts, and allowed the observation of blood vessels and seminiferous tubules.

**Keywords:** *high-resolution, immobilization, magnetic resonance imaging, motion artifact suppression, testis*

## Introduction

Diseases and abnormal conditions of the testis include circulatory disturbance,<sup>1</sup> neoplasm,<sup>2</sup> and infertility (atrophy).<sup>3</sup> The etiology and pathophysiology of testicular disorders are usually studied using various animal models.<sup>4–8</sup> The microstructures of the testis, such as the seminiferous tubules (a region normally 200  $\mu\text{m}$  in diameter) and thin blood vessels, exhibit morphological changes in the diseased or disordered state. High-resolution MR imaging may be able to detect morphological changes in these small structures and provide valuable information on testicular diseases.

Currently, the lack of techniques for testis immobilization has limited the application of high-resolution MR imaging to testicular diseases in both preclinical and clinical studies. Involuntary testicular movements occur at low temperatures by cremaster muscle contractions. In addition, the testis is mobile within the inguinal canal in rare cases and is termed migratory testis. This condition is commonly seen in rats and causes major difficulties in acquiring MR images of the testis in preclinical studies. This is due to the testicular movements that deteriorate image quality by generating motion artifacts and malpositioning the testis. The testis cannot be visualized by high-resolution MR imaging if it deviates from the sensitive area of a small radiofrequency (RF) coil.

Therefore, there is a need for a technique that enables high-resolution MR imaging of the testis that can effectively

and stably immobilize the testis during MR examination. The purpose of this study was to test a novel application of negative pressure suction to immobilize the testis of rats during MR image acquisitions and to determine the pressure levels that assure good positioning and suppress motion artifacts.

## Materials and Methods

### MR scanner

We used a 3 tesla whole body scanner (Signa HDx 3.0T; GE Healthcare, Milwaukee, WI, USA). Radiofrequency transmission was performed using the body coil of the scanner. A 3-cm diameter circular surface coil (Takashima Seisakusho Co., Ltd., Tokyo, Japan) was used as a signal receiver (Fig. 1). We originally developed the coil for clinical research; therefore, the coil was encased (length, width, height: 126  $\times$  50  $\times$  15 mm) in flame-retardant polyvinyl chloride (PVC) according to the International Electrotechnical Commission (IEC) standard (IEC60601-1). We opened a 21-mm aperture through the top and bottom surfaces of the case with the intension of inserting objects and a plastic holder into the aperture. The diameter of the aperture was also designed to keep an appropriate creepage distance according to the IEC standard. This enabled the object to approach the most sensitive area of the RF coil (3 mm downward from the upper surface).

### Animal experiments

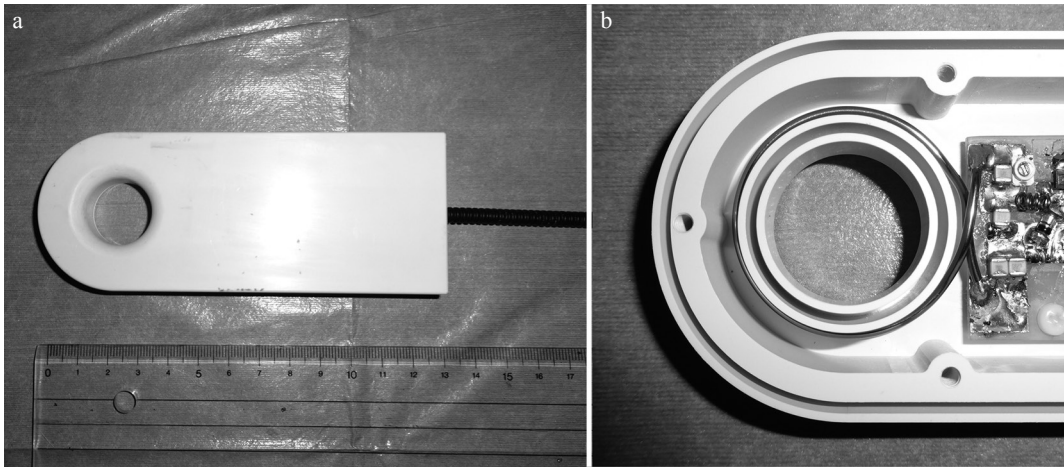
Our Institutional Animal Use and Care Committee approved the protocol. We purchased five male Wistar rats from Japan SLC Inc. (Shizuoka, Japan). After more than 1 week of acclimatization, we performed MR examinations on these rats from 10 to 15 weeks of age at that time. Their body weights ranged between 236 and 325 g. We anesthetized each rat using a gas mixture of isoflurane, oxygen, and nitrous oxide. After anesthesia was induced, we immobilized the right testis using the following procedure (Fig. 2): Each rat was placed

Division of Functional Imaging, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

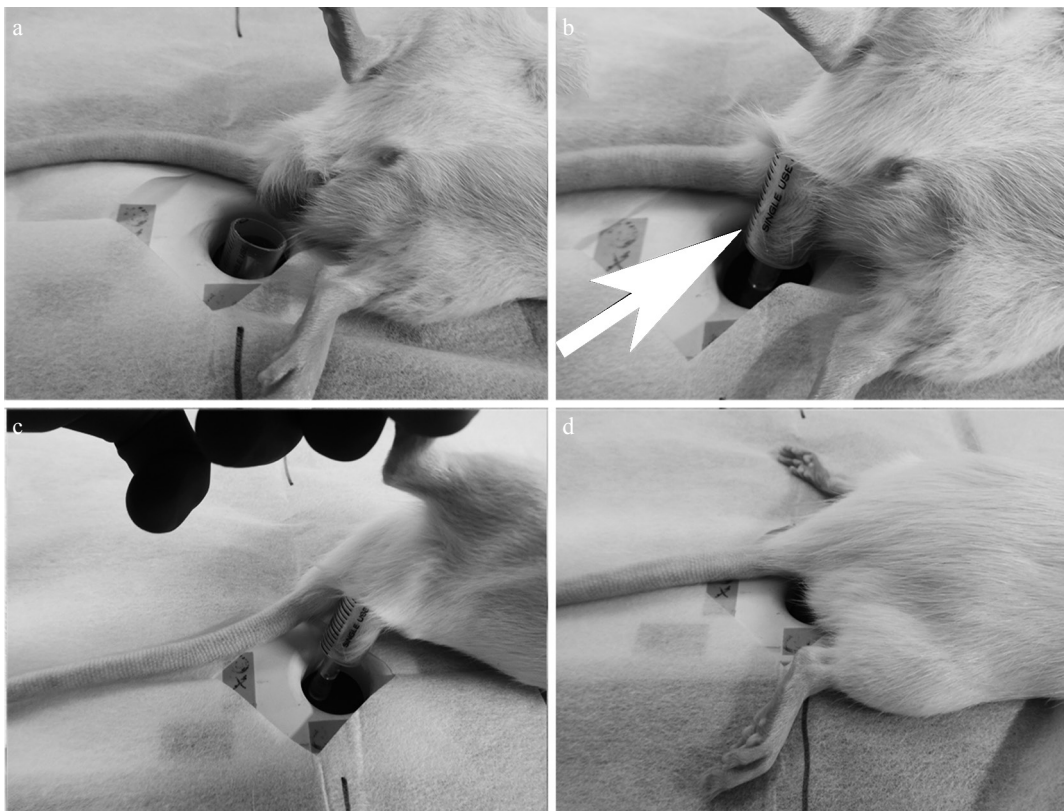
\*Corresponding author, Phone/Fax: +81-4-7134-6832,  
E-mail: masyamag@east.ncc.go.jp

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Received: August 22, 2017 | Accepted: December 8, 2017



**Fig. 1** Small radiofrequency coil. The coil is a circular surface coil 3 cm in diameter (a) and is covered by a flame-retardant polyvinyl chloride (PVC). A 21-mm aperture is made through which an object can be placed in the most sensitive area of the coil (b).



**Fig. 2** Rat positioning. A rat is placed in the left decubitus position (a). The right testis is inserted into a plastic holder (arrow) and vacuumed at pressure of  $-25$  kPa (b). The body of the rat is subsequently rotated with the plastic holder and vacuumed with the testis pointing down into the aperture of the coil (c). Finally, the rat is placed in the prone position (d).

in the left decubitus position (left-side down) with the feet directed towards the magnet, and the testicle was immobilized using a plastic holder (12 mm in diameter and 16 mm in depth) and applying a partial vacuum pressure at minus 25 kPa. The plastic holder was connected in advance to a continuous vacuum pump (Fuji Medical Instruments Co., Ltd, Tokyo, Japan) located outside the magnet room through a flexible polymer tubing. Next, we placed the rat in the prone position and pushed the holder and testicle down in the aperture of the receiver coil. Finally, we inserted the rat onto the magnet. During the MR sessions, we adjusted the pressure values by using a vacuum controller and vacuum gauge that came with the vacuum pump.

### MR imaging

We acquired multiple spin-echo (SE) images using the following parameters: TR 2000 ms; TE 23, 46, 69, and 92 ms; FOV  $40 \times 40$  mm; matrices  $512 \times 256$  (zero-interpolation to  $512 \times 512$ ); receiver bandwidth, 15.63 kHz; slice thickness, 1 mm; and number of excitations, 1. The voxel size was  $0.078 \times 0.078 \times 1$  mm<sup>3</sup>. The acquisition time was 9 minutes and 28 seconds. The phase encoding direction was right-to-left. We acquired multiple SE images because they can provide high signal-to-noise images at a short TE and simultaneously can display various tissue contrast at different TEs. We repeatedly performed multiple SE acquisitions by increasing the vacuum pressure in a step-wise

manner from  $-25$  to  $-10$ ,  $-5$  and  $0$  kPa. In addition, we repeated the same SE acquisitions, but without suction of the specimen to the plastic holder. We inspected the bilateral testes for injuries immediately after the MR sessions.

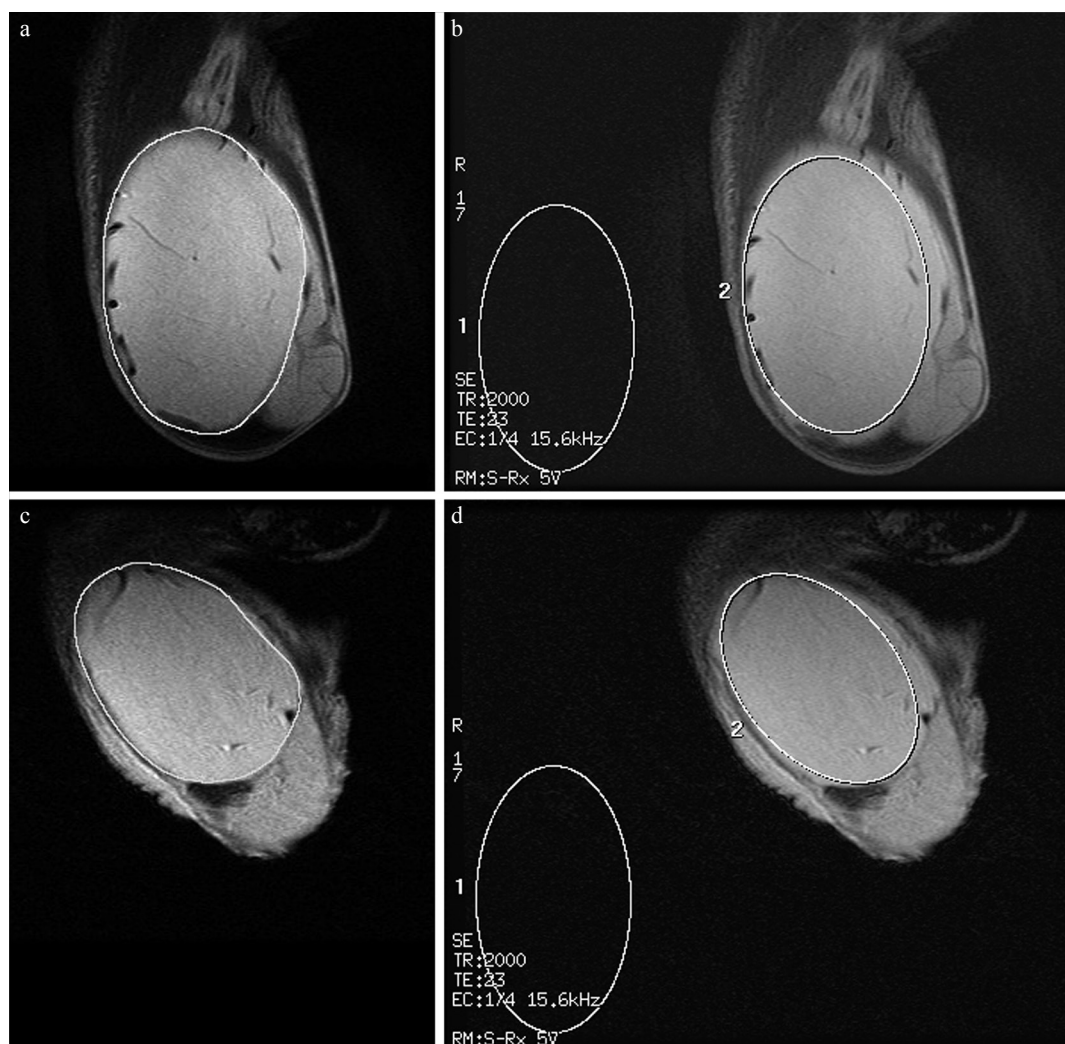
### Image analysis

We evaluated the acquired images using the viewer from the scanner and software (ImageJ, version 1.51j8; National Institute of Health, Bethesda, MD, USA). First, one of the authors who is a well-experienced radiologist qualitatively evaluated the presence or absence of motion artifacts in the SE image series while we applied various strengths of vacuum pressure. Next, he manually drew ROIs tracing the outer surface of the testis, measured the mass of the ROIs by using a function of ImageJ, and evaluated the shift of mass in the vertical and horizontal directions after changing the vacuum pressure. He then placed

another set of spherical ROIs on the testis as well as on the background of the SE images with a TE of 23 ms by using the manufacturer-supplied viewer. We measured the mean signal intensity from the ROIs of the testis and the standard deviations (SDs) of the background noise and calculated the signal-to-noise ratios (SNRs). Figure 3 shows representative ROIs. We compared the SNR values at vacuum pressures of  $-10$ ,  $-5$ , and  $0$  kPa relative to those at a vacuum pressure of  $-25$  kPa.

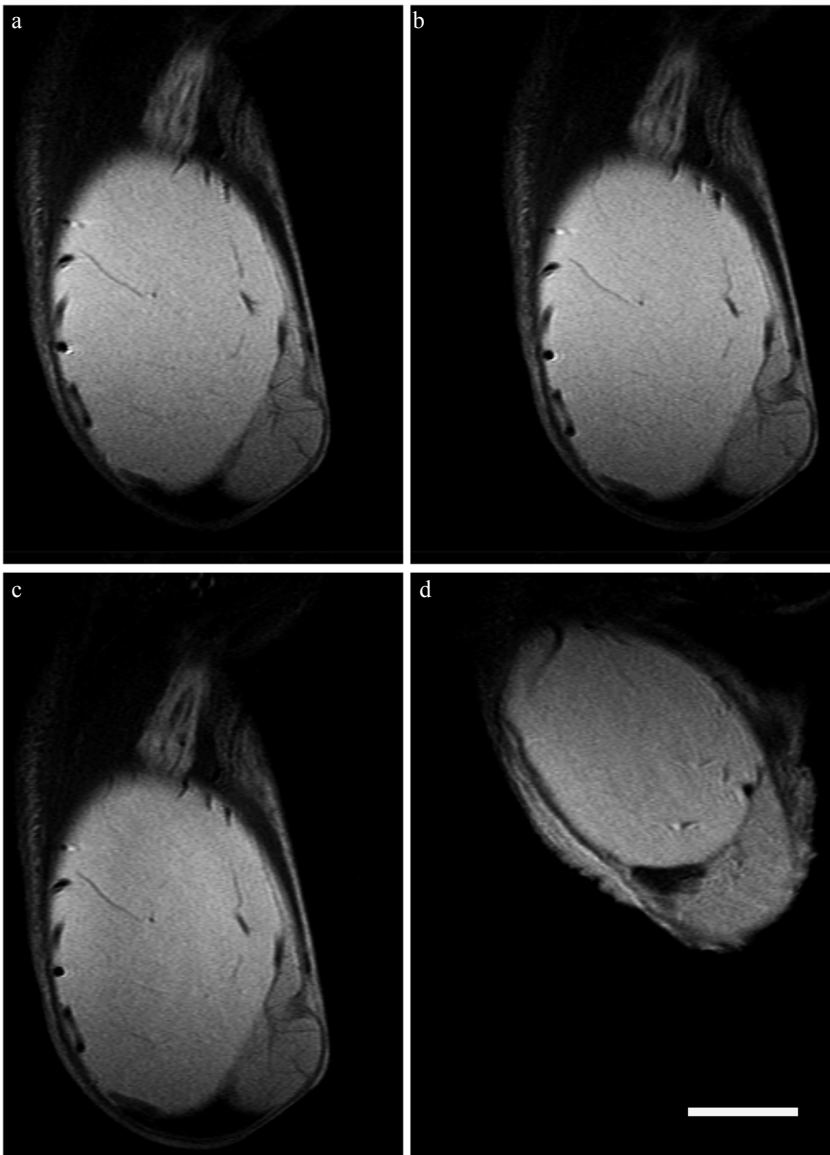
### Statistical tests

We compared the difference in the shift of mass among images for specimens under vacuum pressures of  $-25$ ,  $-10$ ,  $-5$ , and  $0$  kPa using Tukey's test. We also compared the difference in the relative SNR values among the images that were acquired at vacuum pressures of  $-10$ ,  $-5$ ,  $0$  kPa. We used a commercially available software (IBM SPSS



**Fig. 3** Representative ROIs on the testis, which is downwardly immobilized (**a** and **b**) and moves upwardly (**c** and **d**). Manually drawn lines along the outer surface in (**a** and **c**) were used to determine the mass of the testis by using a function of ImageJ (version 1.51j8; National Institute of Health, Bethesda, MD, USA). Spherical ROIs (**b** and **d**) were used to measure mean signal intensity of the testis and standard deviation of the background noise by the manufacturer-supplied viewer.





**Fig. 4** Spin-echo images of the testis (TE = 23 ms). The testis is downwardly immobilized at partial vacuum pressures of  $-25$ ,  $-10$ , and  $-5$  kPa. (**a-c**, respectively). Without vacuum (**d**), the testis moves upwardly. In addition, motion artifacts are noted. Bar represents 5 mm.

Statistics, version 20; IBM Corporation, Armonk, NY, USA) and set the level of significance at  $P < 0.05$ .

## Results

The duration between the induction of anesthesia and the start of MR image acquisitions was typically 20 min. It took approximately 5 min (ranging from 3 to 6 min) to position the testis. When the vacuum was turned on, the testis was downwardly immobilized in all cases. We observed blood vessels on the surface of the testis and those that were deep in the parenchyma (Fig. 4). Although we did not observe motion artifacts on MR images acquired at vacuum pressures of  $-25$  and  $-10$  kPa in any rats, we observed an artifact in 1 of the 5 rats at  $-5$  kPa. However, when the vacuum was turned off (i.e., vacuum pressure of 0 kPa), we observed

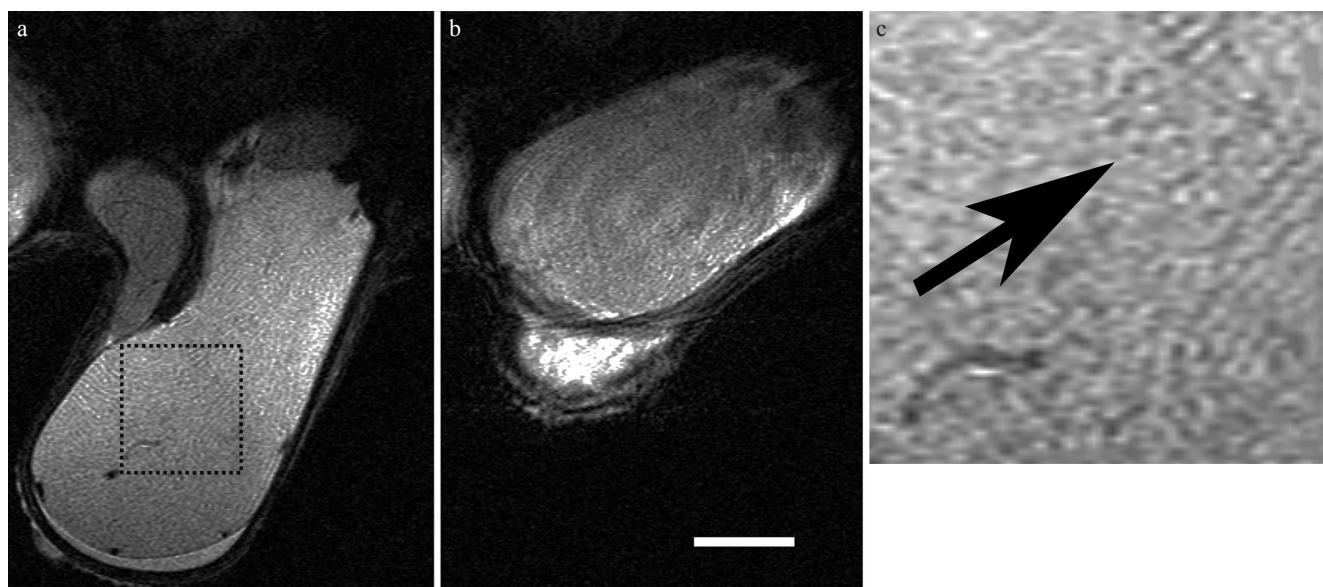
motion artifacts in all rats. Furthermore, the mass of the ROIs was vertically (upwardly) and horizontally shifted at  $5.01 \pm 3.81$  mm (average  $\pm$  SD) and  $1.5 \pm 1.38$  mm from those at  $-25$  kPa. The relative SNR values were reduced to  $0.85 \pm 0.13$  without suction compared to those with suction at  $-25$  kPa. Table 1 summarizes the results. For longer echo-time images, we also observed cross-sections of the seminiferous tubules which were appeared as tubular structures with lower signals at the periphery and higher signals at the center (Fig. 5). On gross inspection, we did not detect any trauma on the vacuumed testis (average size was  $19.9 \times 10.1 \times 9.4$  mm).

## Discussion

Our results indicate that negative pressure suction can immobilize the testis during MRI examinations. It can also

**Table 1** Incidences of motion artifact, shifts of the mass of testis, and relative signal-to-noise ratio (SNR) values among various vacuum pressures

Vacuum pressure [kPa]	Motion artifact	Shift of the mass of testis		Relative SNR [Arbitrary unit]
		Vertical direction [mm]	Horizontal direction [mm]	
-25	0/5	0	0	1
-10	0/5	$-0.08 \pm 0.37$	$0.13 \pm 0.08$	$0.99 \pm 0.03$
-5	1/5	$-0.16 \pm 0.64^*$	$0.23 \pm 0.13$	$0.98 \pm 0.02$
0 (without suction)	5/5	$5.01 \pm 3.81^*$	$1.5 \pm 1.38^*$	$0.85 \pm 0.13^*$

\* $P < 0.05$ .**Fig. 5** Spin-echo images of the testis (TE = 69 ms). The testis is immobilized at a partial vacuum pressure of -10 kPa (a). Without vacuum (b), the testis moves upwardly. In addition, motion artifacts are seen. Bar represents 5 mm. A magnified view (c) of the testicular parenchyma in the dotted lines in (a) shows some transverse sections of seminiferous tubules (arrow).

hold the testis in the right position within the most sensitive area of the surface coil. Using this technique, we were able to acquire high-resolution MR images of the testis with high SNRs with suppressed motion artifacts. From these artifact-suppressed MR images, we were able to observe fine structures in the testis such as thin blood vessels. For longer echo-time images, we also observed cross-sections of the seminiferous tubules in some areas. They showed similar appearances to those previously reported by Yamaguchi et al. at 4.7 tesla.<sup>6</sup> Heavier  $T_2$ -weighting may have allowed a greater contrast between the outer and inner parts of the seminiferous tubules.<sup>6</sup> While partial volume effect can alter the appearance of the seminiferous tubules, it is an unlikely scenario that the tubular structure we observed was artifact displayed on a low spatial resolution image because we have observed the shrinkage and even disappearance of these tubular structures in some artificial testicular disorders in other MR studies with equivalent spatial resolution.<sup>9</sup>

Immobilization techniques using partial vacuum have already been reported in clinics for many parts of the organs except for the testis. Devices for gastrointestinal endoscopy<sup>10</sup> as well as devices for mammography<sup>11,12</sup> are some examples. However, to our knowledge, these techniques have not yet been applied to the testis for the purpose of high-resolution MR imaging.

Previous studies have described various techniques to immobilize the testis such as securing the testis on a purpose-made cradle and applying a piece of tape around the neck of the scrotum.<sup>4,13</sup> Our proposed technique is another approach to immobilize the testis. Our technique is advantageous due to its simplicity, stability, and reproducibility. For example, it only takes several minutes to position the testis after which the testis is held in position for approximately 30 minutes for MR imaging (i.e., 9 min 28 sec  $\times$  3 consecutive SE acquisitions). As shown in Figure 4, the intermeasurement variations in the testis positions were minimal when applying -25 to -5 kPa partial vacuum pressure.

Furthermore, the pressure from the partial vacuum was easily controlled using a vacuum controller; therefore, the examiner was able to secure the testis *in situ* without pulling out the rat body from the magnet. Testicular movements during MR imaging can be controlled easily by further securing the testis.

Since we did not detect major injury to the testis immediately after the completion of the MR examinations, our vacuum method does not cause acute damages to the testicular tissues. However, for future studies, the long term safety of this technique should be investigated. Since our vacuum technique was feasible for high-resolution MR imaging of rat testis, it may, after optimizing the configuration of the RF coil and holder, have potential applications for MR imaging of the human testis.

This paper has the following limitation: While negative pressure suction technique may be applicable for various preclinical MR studies of the testis, including diffusion-weighted MR imaging, perfusion MR imaging, and MR spectroscopy, we did not investigate how negative pressure suction can affect water diffusion, perfusion, as well as the metabolism of the testis. Further investigations are still needed to elucidate the effect of negative pressure suction on testis physiology and metabolism.

## Conclusions

We developed and tested a novel application of negative pressure suction to immobilize the testis of rats for MR image acquisition and found that a suction pressure of  $-5$  kPa can effectively and stably hold the testis in the sensitive area of a small RF coil. Motion artifacts were effectively suppressed at pressures of  $-10$  kPa and, as a result, thin vasculatures and seminiferous tubules could be observed.

## Acknowledgments

This study was supported in part by The National Cancer Center Research and Development Fund, grants from the Foundation for Promotion of Cancer Research in Japan and from the Takeda Science Foundation, to MY.

## Conflicts of Interest

The authors declare no conflicts of interest.

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