

Magnetic resonance imaging for the study of ovarian follicles in the mouse

A.P. Stephenson^a, D.J. Tyler^b, C.A. Carr^b, S.A. Williams^{a,*}

^a Nuffield Department of Obstetrics & Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, UK

^b Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, UK

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Abstract

Additional tools to analyze follicle development would be highly advantageous because current methods require sacrifice of animals at specific times and time-consuming sectioning of tissues for histologic analysis. Magnetic resonance imaging (MRI) may provide a less involved, faster and more cost-effective method to analyze follicles in whole ovaries. Fixed ovaries were collected at different stages of the estrus cycle and after stimulation with gonadotrophins (24 and 48 h post pregnant mares serum (PMSG), and 10 and 24 h post human chorionic gonadotrophin (hCG)) with or without administration of the contrast agent gadodiamide. The MR images were generated using a vertical-bore, 11.7 Tesla MR system. Analysis of the MR images revealed large antral follicles in fixed ovaries with the oocyte and cumulus mass identifiable within preovulatory follicles. The use of gadodiamide had no impact on the quality of MR images obtained. The fixed ovaries were paraffin embedded, sectioned, and hematoxylin stained. Follicles were counted using the MR images and the histology sections. Preovulatory follicle numbers determined using MR images were comparable to those using histology; however counts of smaller follicles were inconsistent. MRI of gonadotrophin-stimulated ovaries *in situ* did not reveal discernable ovarian structures. Therefore, MRI is a useful tool for studying whole fixed ovaries leaving the ovary intact for additional analyses or for selection of samples based on morphology. The MRI is also useful for identifying preovulatory follicles, although analysis of smaller follicles is not possible, and thus the potential exists for cyst analysis in mouse models of polycystic ovarian syndrome (PCOS).

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1. Introduction

To investigate the structural and functional changes that occur in the ovary as ovarian follicles develop, clear imaging of the ovaries is required. These images can be used to determine follicle numbers, a routine measure of follicle development, and are useful to gain insight into the dynamics of follicle development [1,2].

Although highly valuable for the data generated, current methods of follicle counting are time-consuming, with tissues requiring embedding, sectioning, staining and mounting prior to analysis. Moreover, in assessing follicle numbers using this technique, compromises have to be made because unless every section is analyzed, the counts generated provide only a representative sample of the follicle numbers actually present.

In mice, although new methods of ascertaining follicle numbers have been explored to improve accuracy and reliability of results [3], they still rely on histologic sections. MRI is increasingly used in medicine and research. If MR images of whole ovaries could allow

* Corresponding author. Tel.: +44 (0) 1865 221014; Fax: +44 (0) 1865 769141.

E-mail address: suzannah.williams@obs-gyn.ox.ac.uk (S. Williams).

follicle numbers to be ascertained, this would both reduce the time required to collect the data and also leave the tissue intact for alternate analyses. Potentially, it might also be more accurate than histologic analysis of selected sections because the data obtained is from the entire organ. A previous study of MRI analysis of *ex vivo* dog ovaries concluded that follicle and corpora lutea (CL) counts were inaccurate [4]. However, MRI technology continues to improve and a higher field strength MRI scanner, as used to produce detailed images of rat hearts [5,6], may improve analyses of ovarian follicles.

MRI has been used *in vivo* to determine the size of ovarian tumors in mice [7,8], the effect of drugs on the size of ovaries [9] and the growth of ovarian grafts transplanted into hind limbs of nude mice [10]. Attempts have been made to visualize individual follicles *in vivo* in rats using MRI, but images generated using a 4.7 Tesla spectrometer were not successful [11–13]. However, follicle growth measurements have been achieved in human ovarian tissue xenotransplanted into the back muscle and beneath the kidney capsule of mice [14]. Therefore, with the use of higher field strength MRI scanners, it may be possible to image follicles *in vivo*.

This paper explores the potential of high field strength MRI to visualize mouse ovarian follicles in whole ovaries at different stages of development.

2. Materials and methods

2.1. Mice

Ovaries at each stage of the estrous cycles were collected from mice of a mixed genetic background (8–10 wks old with ovaries weighing 4.5–4.9 mg). HsdICR(CD-1) mice age 6 wks weighing 20–22 g were used for the superovulation analyses. All experiments were carried out under authorization of the Local Ethical Review Board and the Home Office.

2.2. Collection of ovaries at each stage of the estrous cycle

To obtain ovaries at each stage of the estrous cycle, vaginal smears were collected daily from female mice for 1–2 wks and evaluated. The smear was obtained by rinsing the vagina with $\sim 100 \mu\text{L}$ 0.9% sodium chloride and this was smeared onto a slide and air-dried. The smear containing vaginal cells was fixed in absolute ethanol for 8 min and stained with Giemsa. Cells were evaluated under a light microscope and samples classified to one of the four stages of the estrous cycle [15]. Diestrus vaginal

smears were determined by the presence of high numbers of leukocytes, during proestrus small nucleated epithelial cells were present, in estrus large numbers of cornified epithelia cells were observed and in metestrus, leukocytes are also present. Mice were sacrificed at each day of the cycle, ovaries collected, fixed in 10% buffered formalin for 8 h, and transferred to 70% ethanol until embedded in agarose.

2.3. Follicle stimulation and synchronization with gonadotrophins

To analyze follicle development, mice were stimulated with gonadotrophins [2]. Mice were injected with 5 IU of pregnant mare's serum gonadotrophin (PMSG; Centaur Services, Castle Cary, UK) and 46 h later injected with 5 IU of human chorionic gonadotrophin (hCG; Chorulon, Centaur Services) to induce ovulation. Mice were analyzed at either 24 h post PMSG, 48 h post PMSG, 56 h post PMSG + 10 h post hCG, or 72 h post PMSG + 24 h post hCG.

2.4. MRI acquisitions

MRI was performed using a vertical-bore, 11.7 Tesla MR system (Oxford Instruments, Abingdon, UK) interfaced to a Bruker console running Paravision 2.1.1 (Bruker Biomedical, Ettlingen, Germany).

For image acquisition of fixed ovaries, a 13-mm quadrature-driven birdcage coil (Rapid Biomedical, Würzburg, Germany) and a 3D fast gradient echo sequence were used (TE/TR 7.5/30 ms; field of view $12.8 \times 12.8 \times 12.8 \text{ mm}$; matrix size, $512 \times 512 \times 512$; voxel size $25 \times 25 \times 25 \mu\text{m}$; 6 averages; total acquisition time 13 h) [16]. Images $25 \mu\text{m}$ apart were collected.

For *in situ* acquisitions, a 28-mm quadrature-driven birdcage coil (Rapid Biomedical, Würzburg, Germany) and a 3D fast gradient echo sequence were used (TE/TR 2.7/10 ms; field of view $25.6 \times 25.6 \times 12.8 \text{ mm}$; matrix size, $256 \times 256 \times 128$; voxel size $100 \times 100 \times 100 \mu\text{m}$; 8 averages; total acquisition time 44 min). Following acquisition, zero-filling was performed to yield images with a $100 \mu\text{m}$ isotropic resolution [16].

2.5. Contrast agent

To assess the impact of the contrast agent gadodiamide on imaging ovaries, one mouse at each time point was injected subcutaneously with $25 \mu\text{L}$ of 0.5 mmol/mL gadodiamide (Omniscan; GE Healthcare, Slough, UK) 30 min before sacrifice. Subcutaneous injection of the contrast agent was chosen as the route of administration since this technique in the mouse

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