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Mini-Review

Imaging the ovary

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Aaron Hsueh is an ovarian physiologist and has published in the field for decades, with over 380 refereed papers. His laboratory has contributed to the understanding of ovarian follicle growth and atresia, intraovarian mechanisms of oocyte maturation, and autocrine/paracrine regulation of early embryonic development, and established the Ovarian Kaleidoscope Database.

KEY MESSAGE

The ovary is a unique organ with hormonally regulated folliculogenesis, follicle rupture, luteal formation/regression and associated vasculature changes, leading to tissue remodelling during each reproductive cycle. Advances in ovarian imaging techniques provide a new understanding of folliculogenesis and vasculature to allow better diagnosis and treatment of ovarian diseases.

A B S T R A C T

During each reproductive cycle, the ovary exhibits tissue remodelling and cyclic vasculature changes associated with hormonally regulated folliculogenesis, follicle rupture, luteal formation and regression. However, the relationships among different types of follicles and corpora lutea are unclear, and the role of ovarian vasculature in folliculogenesis and luteal dynamics has not been extensively investigated. Understanding of ovarian physiology and pathophysiology relies upon elucidation of ovarian morphology and architecture. This paper summarizes the literature on traditional approaches to the imaging of ovarian structures and discusses recent advances in ovarian imaging. Traditional in-vivo ultrasound, together with histological and electron microscopic approaches provide detailed views of the ovary at organ, tissue and molecular levels. However, in-vivo imaging is limited to antral and larger follicles whereas histological imaging is mainly two-dimensional in nature. Also discussed are emerging approaches in the use of near-infrared fluorophores to image follicles in live animals to detect preantral follicles as well as visualizing ovarian structures using CLARITY in fixed whole ovaries to elucidate three-dimensional interrelationships among follicles, corpora lutea and ovarian vasculature. Advances in ovarian imaging techniques provide new understanding of ovarian physiology and allow for the development of better tools to diagnose ovarian pathophysiology.

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Introduction

Using mainly histological analyses of fixed ovarian tissues and ultrasound imaging of ovaries *in vivo*, earlier imaging studies have established the basic framework of ovarian folliculogenesis as well as luteal formation and regression. The ovary contains individual follicles as functional structures, together with corpora lutea, interstitial tissues, innermost medulla and the outmost layer of the surface epithelium. Most of the 800,000 primordial follicles found at birth in human females remain at the dormant stage. After birth, some of these follicles gradually initiate growth (~1000 per month) and progress into primary, secondary and antral stages (Macklon and Fauser, 1999; McGee and Hsueh, 2000). Under the regulation of pituitary gonadotrophins, follicles start growing, with most of them becoming atretic at the early antral stage. Most of the ~20 early antral follicles degenerate during the early follicular phase in women and only one reaches the pre-ovulatory stage. Regulating female reproductive organs by secreting ovarian steroids, the pre-ovulatory follicle eventually ruptures to release the mature egg for fertilization and propagation of the species (Macklon and Fauser, 1999; McGee and Hsueh, 2000). After rupture, the pre-ovulatory follicle undergoes luteinization and secretes progesterone to maintain pregnancy, followed by luteal regression if no pregnancy is established. In adult mammals, the ovary and uterus are the only two organs undergoing hormonally regulated neo-angiogenesis, which is otherwise only found during tumorigenesis (Hazzard and Stouffer, 2000). Cyclic remodelling of ovarian structures and vasculature takes place during each reproductive cycle. This review summarizes traditional approaches to in-vivo imaging of antral/pre-ovulatory follicles, together with two-dimensional histological analyses of relationships among follicles and corpora lutea, followed by recent advances in monitoring preantral follicles using near-infrared imaging in live animals and three-dimensional analyses of relationships among follicles and corpora lutea using a CLARITY approach.

Real-time live imaging of the ovary: from ultrasound to near-infrared imaging

Different approaches have been used to perform real-time ovary imaging in patients and animals. These technologies, including ultrasound, magnetic resonance imaging (MRI), computed tomography (CT), optical coherence tomography (OCT), fluorescence molecular tomography (FMT) and near-infrared imaging, have been developed in mouse models to enhance the ability of clinicians to diagnose ovarian diseases. Each real-time imaging modality has its own limitations and strengths in imaging the ovary, as summarized in **Table 1**.

Ultrasound is the most widely used in-vivo approach to ovarian imaging; it uses sound waves to create an image on a video screen. Sound waves are emitted from a small probe placed transvaginally in the woman or on the surface of her abdomen to create echoes from the ovaries. The same probe detects the echoes that bounce back and a computer translates the pattern of echoes into a picture. Because of the unique position of the ovary inside the body, vaginal ultrasound provides close imaging of ovarian structures for detecting large antral follicles, ovarian tumours and fluid-filled cysts. With the transvaginal route, high-frequency ultrasound probes (>6 MHz), which have a better spatial resolution but less examination depth, are useful because the presence of fatty tissue does not interfere with imaging (Balen et al., 2003). However, it is difficult to image preantral (secondary, primary and primordial) follicles in women using transvaginal ultrasound, making this approach inadequate for checking residual follicles in patients with diminished ovarian reserve (Levi et al., 2001). As shown in **Figure 1A**, B-mode ultrasonography for imaging of the ovary allowed visualization of large follicles with an antral cavity in an adult mouse but smaller follicles without an antrum were not detectable. Furthermore, the power Doppler ultrasound imaging can be applied to evaluate mouse ovarian vasculature with comparable resolution (Migone et al., 2016).

In addition to ultrasonography, CT and MRI scans represent advances in the live imaging field. The CT scan uses an X-ray procedure to produce cross-sectional images of the body. A CT scanner takes many pictures as it rotates around the body, before digitally transforming images into multiple slices of body images. CT scans do not show small ovarian follicles, but they can detect large follicles and tumours (Ocak et al., 2015). Using vaginal ultrasound provides similar resolution but at a lower cost, so the CT approach is not routinely used for follicle imaging. As shown in **Figure 1B**, a CT scan of an adult mouse ovary showed poor differentiation of the ovary from surrounding organs.

MRI scans use radio waves and strong magnets instead of X-rays. The energy from the radio waves is absorbed and a computer translates the pattern of radio waves given off by tissues into a detailed image of parts of the body. In addition, a contrast material might be injected to refine scanned images. As shown in **Figure 1C**, MRI was used for imaging of an adult mouse ovary *in vivo* (Ocak et al., 2015). Although MRI provides clear imaging of large follicles in the ovary *in vivo*, this approach has not been routinely applied to ovarian follicle detection in the clinic due to the difficulties involved in accessing the imaging probes to the ovary.

Optical coherence tomography (OCT) imaging *in vivo*, based on near-infrared light (**Figure 1D**), has been adopted for evaluation of mouse ovaries (Burton et al., 2015). The use of relatively long wavelength light provides cross-sectional views of the subsurface microstructure of biological tissues (Schmitt, 1999). To decrease the effect of tissue motion (breathing or muscle contraction) during live imaging using OCT, an OCT system with higher imaging speed has been recommended (Burton et al., 2015). The OCT approach, however, suffers from the design of practical scanning systems for clinical application.

As an experimental tool, intravital multiphoton microscopy, a fluorescence imaging technique that allows imaging of living tissue up to about 1 mm in depth (**Figure 1E**), was recently used to monitor blood flow of individual vessels and the thickness of the apical follicle wall during ovulation in murine models *in vivo* (Migone et al., 2016). Constriction of apical vessels was found to occur within hours preceding follicle rupture (Migone et al., 2016). Furthermore, preventing the periovulatory rise in the expression of the vasoconstrictor endothelin 2 inhibited ovulation whereas infusion of vasoconstrictors (either endothelin 2 or angiotensin 2) into the bursa restored ovulation (Migone et al., 2016).

A similar intravital window approach for live imaging *in situ* was used to enable longitudinal microscopic analyses of ovarian morphology and orthotopic tumour invasion (Bochner et al., 2015). The morphological structures of the murine ovarian cortex were examined together with cellular dynamics after gonadotrophin treatment *in vivo*. Of interest is the longitudinal imaging of the orthotopic tumour formation *in vivo* by following infiltration of tumour cells through the

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