In vivo Dynamic Volumetric Imaging of Mouse Testis and Epididymis

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The implementation of live imaging in reproductive research is crucial for studying the physiological dynamics. Sperm transport is a highly dynamic process regulated by tubular contractions and luminal flows within the male reproductive tract. However, due to the lack of imaging techniques to capture these dynamics *in vivo*, there is little information on the physiological and biomechanical regulation of sperm transport through the male reproductive tract. Towards this technical limitation, we develop a functional *in vivo* imaging approach using optical coherence tomography, enabling live, label-free, depth-resolved, three-dimensional, high-resolution visualization of the mouse testis and epididymis. With this approach, we spatiotemporally captured tubular contractility in mouse testis and epididymis, as well as microstructures of these reproductive organs. Our findings demonstrated that the contraction frequency varies significantly depending on the epididymal regions, suggesting the spatial regulation of epididymal contractility. Furthermore, we implemented quantitative measurements of the contraction wave and luminal transport through the epididymal duct, revealing the physiological dynamics within the male reproductive tract. The results show that the contraction wave propagates along the epididymal duct and the wave propagation velocity was estimated *in vivo*. In conclusion, this is the first study to develop intravital dynamic volumetric imaging of the male reproductive tract, which allows for quantitative analysis of the dynamics associated with sperm transport. Our findings set a platform for various studies investigating normal and abnormal male reproductive physiology as well as the pharmacological and environmental effects on reproductive functions in mouse models, ultimately contributing to a comprehensive understanding of male reproductive disorders.

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In Vivo Dynamics of Mouse Ovulation and Oocyte Transport

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Ovulation is a highly dynamic reproductive event where mature oocytes are released from the ovary into the periovarian space, followed by their transfer to the oviduct. This process is critical to human fertility: ovulation-related disorders are the leading causes of female infertility, accounting for 32% of cases. Therefore, much effort has been put into understanding the genetic and endocrine regulations of ovulatory processes. However, limited imaging access to the site of ovulation has left unclear how oocytes behave within the ovulating follicle and how they travel to the site of fertilization after ovulation. The objective of this study was to investigate the dynamic regulation of ovulation and subsequent oocyte transport using mouse models. Toward this aim, we develop a novel approach for *in vivo* 3D imaging of ovarian tissues using optical coherence tomography that allows spatiotemporal analysis of mouse ovulation within the periovarian space. With this approach, we volumetrically and quantitatively capture both the process of ovulation as well as the transfer of ovulated oocytes through the periovarian space into the infundibulum, the section of the oviduct closest to the ovary. We observed a slow, gradual release of the oocyte surrounded by the cumulus cell mass after follicular rupture, which coincides with the reduction in follicular volume. The oocyte shows the highest velocity and becomes stretched into an oval shape as it exits the follicle, suggesting that the oocyte may be squeezed through a follicular opening. After ovulation, spatial tracking of the oocyte reveals that the ovulated oocyte remains at the ovulation site for approximately 25 minutes. After leaving the site of ovulation, the oocyte shows directional movement into the infundibulum and reaches the ampulla within a few minutes. *In vivo* volumetric imaging reveals additional intriguing observations which provide new insight into oocyte transport mechanisms. During ovulation, the infundibulum repeatedly pumps oviductal fluid into the periovarian space. This finding aligns with micro-computed tomography analysis of the ovarian bursa, a membrane surrounding the periovarian space, showing dramatic expansion during ovulation. Another intriguing finding is that spatial tracking of scatters such as loose cumulus cells and mucus demonstrated bidirectional periovarian flow inside the ovarian bursa. Interestingly, the velocity of periovarian flow was significantly higher during ovulation than before ovulation. These results suggest that the oviductal pumping may cause the ovarian bursa to expand during ovulation and potentially generates the periovarian flow. To investigate whether the oviductal pumping plays a role in ovulated oocyte transport, we by inhibited oviductal smooth muscle contraction during ovulation. The mice treated with the inhibitor showed a lower number of oocytes successfully transported to the ampulla and a higher number of oocytes remaining inside the ovarian bursa, indicating that inhibition of oviductal contraction caused retention of ovulated oocytes in the ovarian bursa. Together, our findings demonstrate that pumping induced by oviductal contraction contributes to oocyte transport from the site of ovulation to the site of fertilization. In conclusion, this study reveals the physiological dynamics of ovulation, captures never-before-seen oocyte behaviors, and proposes a potential mechanism for ovulated oocyte transport through the periovarian space into the oviduct.