Understanding the Role of Cilia in Preimplantation Oviductal Transport.

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Motile cilia lining the lumen of the oviduct are known to play an important role in reproduction. Motile ciliopathies, such as primary ciliary dyskinesia, are associated with subfertility and infertility, as well as being linked to a higher incidence of ectopic pregnancies. However, the specific role of cilia in the oviduct remains unknown. Traditionally, it has been presumed that cilia facilitate the directional transfer of eggs and embryos through the oviduct to the uterus for implantation. However, imaging of the reproductive organs in their native state is challenging, and there is little information on the dynamic aspects of cilia transport processes in the oviduct. Therefore, this study aims to investigate the role of cilia in preimplantation oviductal transport through genetic and live imaging approaches.

To determine the potential role of cilia in fertility, we generated a mouse model with conditional deletion of ciliary motor protein-encoding gene *Dnah5* in the oviductal epithelium and performed a six-month fertility trial. A phenotype of subfertility was established, with litter sizes being significantly reduced in *Dnah5* cKO mice.

To confirm the loss of ciliary function, oviductal mucosal surfaces were visualized with digital videomicroscopy. The location and frequency of beating cilia were analyzed by fast Fourier transformation of the pixel intensity fluctuations. The percentage area of actively beating cilia decreased from nearly full coverage to small, isolated patches in *Dnah5* cKO oviducts.

In vivo 3D imaging of the reproductive organs using optical coherence tomography revealed that loss of *Dnah5* in oviductal epithelial cells leads to impaired egg transport, with 50% of ovulated eggs trapped in the ovarian bursa cavity at 0.5 days post coitus compared to control mice, where all eggs were successfully transported to the ampulla. These results suggest that oviductal cilia play a significant role in maintaining timed, preimplantation transport.

Additionally, we hypothesized that cilia drive circulatory flows in the ampulla by the non-uniform spatial distribution of motile cilia throughout the oviductal mucosa. To test this hypothesis, we investigated the distribution of cilia through a combination of light-sheet and confocal fluorescence microscopies using acetylated alpha-tubulin immunostaining to label the cilia.

Volumetric light-sheet microscopy revealed that the mucosal lining of the infundibulum, upper ampulla, and lower ampulla was densely ciliated, while the isthmus contained pockets of ciliated cells. Furthermore, light-sheet rendering revealed a dramatic reduction in mucosal fold number within the lower ampulla where cumulus oocyte complexes are known to display circulatory movements.

Confocal microscopy of oviductal cross-sections at specific locations revealed non-uniform radial distribution of cilia. Cilia coverage is uniform along the infundibulum region but dramatically changes in the upper and lower ampulla, such that the luminal surface of the outer curvature is more densely ciliated than the inner curvature. Interestingly, this distinct ciliary pattern coincides with the portion of the ampulla where cumulus oocyte complexes are known to display circulatory movements. These results support the hypothesis that the morphology and ciliary distribution of the lower ampulla may play a role in facilitating the circulatory flows in this region of the oviduct, which might be critical for luminal mixing and denudation of cumulus oocyte complexes.

This study reveals specific functions of cilia and highlight the important role that cilia play in overall reproduction. This work will contribute to a better understanding of oviduct transport pathologies and could lead to improved management of reproductive ciliopathies.