COMMENTARY

Cilia take the egg on a magic carpet ride

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A critical step in fertilization of eutherian mammals is the "pickup" of oocytes (eggs) by the oviduct (fallopian tube) from the surface of the ovary. In the ovary, each oocyte matures within a follicle, in which it is surrounded by supportive granulosa cells. As the time of ovulation nears, the granulosa cells close to the oocyte transform into cumulus cells, which begin secreting a hydrated elastic extracellular matrix (1). At ovulation, the oocyte plus its cumulus cells and their matrix—the cumulus–oocyte complex (COC)—is released from the ovary and must be pulled into the oviduct, vaulting over a gap between the surface of the ovary and the open end of the oviduct, the infundibulum. Failure of COC pickup can lead to failure of sperm to fertilize the oocyte or, worse, to ectopic implantation of an embryo outside of the uterus. In PNAS, Yuan et al. (2) demonstrate that the carpet of tiny motile cilia that coats the lining of the infundibulum is crucial to successful oocyte pickup and transport into the oviduct.

Yuan et al. (2) created a strain of female mice whose oviducts were largely devoid of cilia. They did this by simultaneously knocking out two clusters of microRNAs (miRs) that, together, are necessary to generate cilia on the epithelial cells of the oviduct; tissue/time-targeted knockout narrowed down the effects of the miRs to the adult oviduct epithelium. Knockout females were infertile, even though they produced normal amounts of oocytes and reproductive hormones. Their infertility resulted, instead, from a failure of oocyte pickup: Yuan et al. (2) observed that oocytes did not enter the oviducts of the knockout mice but instead accumulated in the bursal cavity outside of the oviduct after they had been released from the ovary. Although some knockout female mice retained a reduced number of motile cilia in the oviduct, it is not surprising that those cilia were insufficient to support COC pickup and transport, because the 400-μm diameter of the COC for a single mouse oocyte (1) is 80-fold larger than the 5.1-μm average length of wild-type (WT) cilia (2). Many cilia, working in concert, must be needed to transport the large COC.

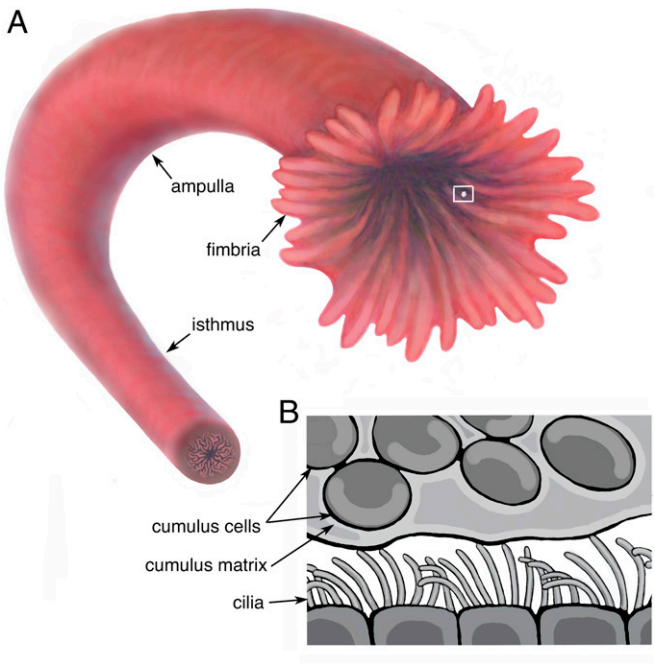

Fig. 1. (*A*) Human oviduct, with fimbriated portion of the infundibulum visible. The oviduct has been cut in midisthmus. A white box surrounds a COC (approximately to scale) being transported into the infundibulum. (*B*) A highly magnified view of the cumulus matrix of the COC interacting with oviductal cilia. Illustrations credit: Rose Gottlieb (artist).

Two distinct factors contribute to the successful pickup of the COC and its transfer into the oviductal lumen by cilia: 1) A coordinated unidirectional sweeping movement produced by a carpet of ciliated cells can "waft" the COC into the oviduct and 2) adhesive interactions between the tips of the cilia and the cumulus matrix of the COC help the cilia hold onto the oocyte as they move it (3). The latter was clearly demonstrated in hamsters, where experimentally

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increasing or decreasing the adhesive interactions resulted in failure to transport COCs into the oviduct (3). The molecules that directly mediate oocyte adhesion to cilia are unknown. The extracellular matrix of the COC consists primarily of the nonsulfated anionic glycosaminoglycan hyaluronan and the proteins inter-α inhibitor (Iαl), pentraxin 3 (PTX3), and tumor necrosis factor–stimulated gene-6 (TSG-6), which play a role in stabilizing the matrix by cross-linking the long, flexible hyaluronan molecules (4). The flexibility and negative charges of hyaluronan molecules contribute high elasticity and extreme softness to the matrix (1), properties that likely play an important role in enabling the carpet of cilia to move the COC along. This phenomenon is reminiscent of the mucociliary apparatus that lines the airways of the respiratory system to clear it of particles in inhaled air. In that apparatus, a blanket of sticky, hydrated, elastic mucus produced by secretory cells in the airways floats on the tips of a carpet of cilia. The mucus traps inhaled particles and the cilia move mucus and particles up to the pharynx to be swallowed (5).

In addition to their critical role in oocyte pickup, oviductal cilia have two additional fertility-promoting functions in female mice: controlling sperm migration to the site of fertilization and transporting the embryo from the site of fertilization in the oviductal ampulla to the uterus for implantation. Yuan et al. (2) observed that both of these functions were reduced, but not eliminated, in the miR-knockout female mice. For example, although the ciliary function of regulating sperm migration up the oviduct to the site of fertilization in the ampulla occurred in the miR knockouts, fewer sperm arrived at the ampulla by 3 h after mating in the knockouts than in the WT females. Control of sperm migration and of embryo transport occur primarily in the isthmus of the oviduct. This region, which lies between the uterotubal junction and the oviductal ampulla, tends to have thicker, more muscular walls and fewer cilia than the ampulla. These anatomical attributes may reflect the roles played by the isthmus in transporting sperm and embryos.

While a carpet of cilia is wafting the COC into the oviduct and then down to the site of fertilization sperm must migrate up the oviduct to meet the oocyte. What enables the two different gametes to move in opposite directions? Oviductal cilia affect sperm migration in one manner by binding to sperm in the lower part of the isthmus. Binding to cilia holds sperm in a reservoir until the periovulatory period (6). Then, sperm are released from cilia by modification and loss of sperm ligands for ciliary receptors (7), combined with hyperactivation of motility (8, 9). Hyperactivation, which deepens flagellar bending, appears to provide sperm with increased leverage to use in order to release from cilia (8, 9). The released sperm must then swim or be transported up to the COCs in the oviductal ampulla. There is evidence that this ciliary function ensures that enough sperm, but not too many, are available to fertilize the oocyte when it enters the oviduct. If too many sperm arrive at the COC, polyspermic fertilization is more likely to occur, resulting in failure of embryonic development (6). If too few sperm arrive at the newly ovulated oocyte, fertilization may not occur because the oocyte has only a short span of time during which it is capable of fertilization (10).

In the periovulatory period, peristalsis of muscles in the isthmus of the oviduct moves mouse sperm up to the ampulla using a shuttling action (11, 12). A second process, rheotaxis, can also guide sperm up the oviduct. In this process, sperm orient and swim upstream against a gentle fluid flow (13). In the cow (*Bos taurus*), for example, sperm are seen within 20-μm-wide microgrooves that line the cervical canal. These microgrooves are lined with ciliated cells and mucus-secreting cells. In transmission electron micrographs, sperm within the microgrooves are seen oriented "upstream," toward the uterus. The orientations of the cilia in the microgrooves indicates that they create a flow downward, toward the vagina, against which the sperm can swim (14). This rheotaxis response to cilia-generated flow can serve to guide sperm to the uterus. In the mouse, there are fewer cilia in the isthmus and lower ampulla than in the infundibulum; the cilia in these regions do not form a solid carpet (15, 16). The fluid flow produced by the cilia might be gentle enough to promote sperm to swim upstream to the ampulla.

In PNAS, Yuan et al. demonstrate that the carpet of tiny motile cilia that coats the lining of the infundibulum is crucial to successful oocyte pickup and transport into the oviduct.

Transporting early embryos from the oviduct to the uterus involves ciliary beating, as well as contractions of smooth muscle layers in the oviduct walls (17). The relative contributions of the cilia and muscles vary among species and are not well understood in humans or mice; however, the results reported by Yuan et al. (2) indicate that ciliary action is only partially necessary for embryo transport in mice.

Altogether, the findings of Yuan et al. (2) support the hypothesis that a carpet of morphologically normal ciliated cells with fully motile cilia is required for pickup of the COC and its transport into the oviduct—at least in the mouse. In addition, their work indicates that oviductal cilia contribute to regulating sperm migration through the oviduct and transporting early embryos to the uterus for implantation and completion of development.

While the findings of Yuan et al. (2) are relevant to human reproductive medicine, there is a noteworthy difference between the reproductive anatomies of women (Fig. 1) and female mice. The rodent ovary is encapsulated by a thin membrane ("bursa") that is fused with the oviductal infundibulum (18). This membrane made it possible for Yuan et al. (2) to find and examine the oocytes that did not enter the oviducts in the knockout mice. In women there is no encapsulating ovarian bursa; this alone could make oocyte pickup more challenging. The large fimbria that surround the opening of the human infundibulum can compensate somewhat for the absence of a bursa, because the cilia-lined fimbria move close to the ovary at ovulation and sweep over its surface (19). If the fimbria fail to catch the COC, it can fall into the peritoneal cavity and be fertilized by sperm that swim out of the infundibular end of the oviduct (20), resulting in abdominal ectopic implantation that can be life-threatening to women (21). Given the intriguing results from Yuan et al. (2), in the future it would be interesting to examine the bursas of knockout mouse females several hours after mating to see if any oocytes in the bursa were fertilized. If any are seen, it may make the mouse a useful model to investigate abdominal ectopic pregnancy.

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