During mammalian reproduction, millions of sperm undertake a journey that only one will finish. This journey to fertilization has been commonly viewed as a race: a sea of sperm competing to get to the egg first.

Yet, Susan Suarez, professor emeritus of biological sciences at Cornell University, and many of her colleagues see things differently. “I certainly don’t think of it as a race,” she says, “but rather as a complex process” involving both sperm and female cells.

Suarez and others have spent decades trying to get a clearer picture of the female reproductive tract’s active role in mammalian conception, which has been largely overlooked compared with that of the sperm.

Suarez partly attributes the focus on sperm to a historic bias toward researching conditions that more often affect males, which led researchers—herself included—to assume that sperm are the essential players in the trip to the egg. In addition, she says, sperm are far easier to collect, observe, and keep alive in the laboratory than the tissues of the female tract.

By doing lab experiments on and observing live animals, researchers are now uncovering bit by bit how female cells interact with sperm at key points on the way to fusion of the sperm and egg—weeding out, preparing, and even storing sperm for fertilization.

Studies in animals such as sheep, mice, and pigs have shown that at each stage—as sperm start in the vagina, move into the cervix, traverse the uterus, then squeeze through a narrow passage into the oviduct called the uterotubal junction (UTJ)—female cells provide biochemical cues as well as put up obstacles to help ensure that only one sperm cell with intact DNA makes it to the egg. Understanding the molecules involved in this process, scientists hope, could offer help for current challenges in livestock breeding, artificial insemination, and human in vitro fertilization (IVF).

Navigating the cervix

Sean Fair, who studies animal reproduction at the University of Limerick, has been puzzled for years by a group of Norwegian sheep.

Sheep are notably difficult to artificially inseminate. The folds in their cervixes are oriented in such a way that semen Healthy sperm preferentially travel up the microgrooves (purple inset) of a sheep cervix as cervical fluid flows down the middle, flushing out immobile cells and pathogens. Credit: Adapted with permission from Reproduction, DOI: 10.1530/REP-18-0595.

Published: January 10, 2022
can’t be placed farther than the entrance of the cervix. That leads, internationally, to poor pregnancy rates for artificial insemination using stored (frozen, then thawed) semen—less than 30% in ewes compared with around 60% for cattle. But in Norway’s small sheep industry, “the farmers themselves are just popping the semen into the vagina, and they get pregnancy rates of 60—70%,” Fair says.

Fair’s group found that neither the skill of the farmers nor the breed of the rams made as much of a difference as the breed of the ewes. Sperm pass through the cervix in more fertile breeds like the Norwegian ewes far better than in others. Fair suspected that what sets these ewes apart lies in their cervical mucus, a liquid made sticky by its sugar-linked mucin proteins.

Much of the time, the mucus serves as a thick barrier to both sperm and pathogens. Yet around ovulation, the cervical mucus changes drastically in volume, viscosity, and composition. Estrogen binds to the epithelial cells that line the cervix and spurs them to produce more fluid. In humans, fluid production ramps up 10-fold around ovulation, increasing to about 700 mg per day from 60 mg at other times. The cervical mucus also becomes thinner as the strings of sugars—mostly O-glycans—sported by mucin proteins attract extra water in the lead-up to ovulation. At the same time, those glycans swap many of their acidic sugars, which include sulfate and sialic acid groups, for more neutral ones. Sperm move better and live longer when traversing this less acidic, thinner cervical fluid.

To figure out how the more fertile sheep breeds ease the passage of sperm, Fair looked closely at the sugars in the ewes’ cervical fluid using a combination of mass spectrometry and liquid chromatography. Comparing Norwegian sheep with other breeds, he found lower levels of particular sialic acids on their O-glycans. These sialic acids bind sperm and inhibit their progression through the cervix, Fair says, though many other factors are likely involved as well.

Fair is also studying how the cervix selects the fittest sperm. Cervical fluid flows gently downward, out of the cervix, flushing out pathogens and dead cells. In mammals, tiny grooves run up and down the inner cervix—just the right size for sperm. Instead of traveling in the middle, sperm swim upstream along these narrow passageways, hugging the cervix’s walls, Fair says. Sperm with abnormal shapes and flagella don’t travel these channels as readily and get flushed back by the outward flow of mucus.

Fair is now applying that selection process to assisted reproduction. Over the past five years, he has worked with bioengineers to design microfluidic devices with channels that model cervical grooves and fluid flow. Human sperm that make it through the device have, overall, more intact DNA, better motility, and better morphology than sperm that don’t.

In 2019, Fair cofounded neoMimix, a start-up to commercialize the device for selecting fitter human sperm. It is being tested at a fertility clinic in Europe. He says the technology could replace current sperm selection methods based on centrifugation and improve the quality of sperm used in infertility treatments.

Opening the junction door
Once past the cervix, uterine contractions help guide sperm to the UTJ—the passageway from the uterus into the oviduct, or the fallopian tube in humans. The UTJ presents a very different obstacle to the pack of sperm and results in a different sort of selectivity.

If the cervix is like a lobby entrance, the UTJ is more like a closet door. It’s smaller and more constricted, and admission appears to be even further restricted biochemically by the female tract.

Studies from the early 2000s showed that mouse sperm engineered to lack any of 15 different proteins could not cross the UTJ, even though all the sperm swam normally and otherwise looked healthy. Why proteins unrelated to swimming help sperm enter the UTJ has been a mystery since then.

Last year, Ying Zhang of Beijing Normal University, Martin Matzuk of Baylor College of Medicine, and co-workers found that sperm’s ability to aggregate may be key to their passage. Using fluorescence imaging of a female mouse’s reproductive tract, the researchers see how normal sperm pack themselves into dense bunches, with heads and tails aligned at the UTJ, and begin to burst through in bright clusters of dozens or more at a time.

“Everyone thought before this point that the sperm go one by one into the oviduct,” Matzuk says. “But based on our work, it looks like they need to hold hands.” Zhang hypothesizes that the sperms’ coordinated tail-beating provides enough collective force to push the UTJ open briefly.

The group then tracked sperm from a mouse engineered to lack a membrane protein called Tex101, one of the 15 required proteins. The sperm filled the uterus all the way up to the UTJ. They did not aggregate, however, and few passed the junction.

Sperm clustering may also be important for other species. Suarez found that bull sperm swim in formation in fluid that mimics the cervical mucus of a cow during the most fertile
period of its cycle. Others saw similar clustering in human sperm.

The authors surmise that clustering at the UTJ could help explain why low sperm counts matter so much to fertility. Without at least a minimum number of sperm—about 20% of the average mouse’s sperm count, Zhang says—no mouse sperm got past the UTJ. An inability to cluster and hold open the UTJ also could be one reason for unexplained infertility in animals and humans even when sperm count is normal and individual sperm cells look typical, Zhang says.

Sperm reservoir

After the effort of squeezing through the UTJ, sperm cells find themselves in the oviduct, the narrow tube that leads to one of the ovaries. Here, rather than push on, the sperm halt their forward movement and instead attach to the cells of the oviduct, as Suarez observed in mice in the 1980s and others observed in larger mammals.

A few years later, Suarez found that bull sperm incubated with cells from the oviduct move and stay functional longer, implying that the oviduct acts as a rest stop in the reproductive tract where sperm are stored, maintained, and released by the epithelial cells when needed for fertilization. Such sperm reservoirs in mammals extend the window for fertilization between mating and ovulation and help prevent more than one sperm from fertilizing an egg at a time, a phenomenon known as polyspermy that can lead to pregnancy complications and loss in mammals.

David Miller specializes in mammalian fertilization research at the University of Illinois Urbana–Champaign, where he studies sperm reservoirs in pigs. Building on research that suggested sperm could bind to oviduct cell sugars, Miller started searching in 2013 for the specific glycans that pig sperm bind to. From an array of 400 glycans, the sperm bound exclusively to two sugar motifs: a simple, three-sugar chain made of galactose, fucose, and glucosamine; and a branched 11-sugar chain.

Miller and his team were impressed by how picky the sperm were for those two structures. “The specificity was really exquisite,” he says. Even very similar sugars were not bound by the sperm. When the researchers went looking for those sugars on the tips of glycoproteins in pig epithelium, they found them on cells lining the oviduct between the UTJ and the site of fertilization but not at the site of fertilization itself.

Pig sperm bind to cells removed from a pig’s oviduct. In the oviduct, these cells allow sperm to survive longer and thus act as a sperm reservoir. Credit: Animal, DOI: 10.1017/S1751731118000526 (CC BY-NC-ND 4.0).

Miller also found that sperm bound to those sugars live longer. He isn’t sure how they survive, but he has some clues. The sole sperm cell that eventually reaches the egg has to go through a multistep, little-understood maturation before it is ready to unite with the egg. Part of that maturation involves a gradual increase in calcium.

Most recently, Miller’s group found that progesterone, a hormone released by the ovaries, can spur sperm cells to detach from oviduct cells by activating a calcium channel on the sperm’s membrane. The channel begins to draw calcium ions into the sperm cell and causes its calcium levels to spike, which triggers the sperm’s tail to beat like a whip, with a more exaggerated and forceful rhythm. That vigorous movement, Miller suspects, helps the sperm undock from the oviduct reservoir and make a break toward the egg.
Progesterone doesn’t affect all the sperm at once, Miller notes. Only about 50% detach at a time, which supports the theory that sperm reservoirs seem to prevent polyspermy—a significant problem with IVF in pigs, he says. He hypothesizes that using sperm that are prebound to oviduct sugars would allow scientists to perform IVF with smaller amounts of higher-quality sperm than currently needed and thus lower the chances of polyspermy.

For artificial insemination, extending sperm lifetimes with glycans from oviduct cells could help mammalian sperm last both inside and outside the oviduct. Sperm attached to beads with these glycans could be inserted directly into live animals and even into humans, although Miller says we’re a long way from that.

These snapshots illustrate just a few of the contributions the female tract makes during the long road to fertilization. Narrow channels, functional fluid flow, and a delicate dance of molecules guide and challenge the sperm. According to Cornell’s Suarez, future insights into the dynamism and complexity of the female reproductive environment will likely come from using genetic editing and imaging to study living animal models. Understanding how sperm and female cells interact inside the female tract may help improve artificial and assisted reproduction in humans.

Louisa Dalton is a freelance contributor to Chemical & Engineering News, an independent news publication of the American Chemical Society.