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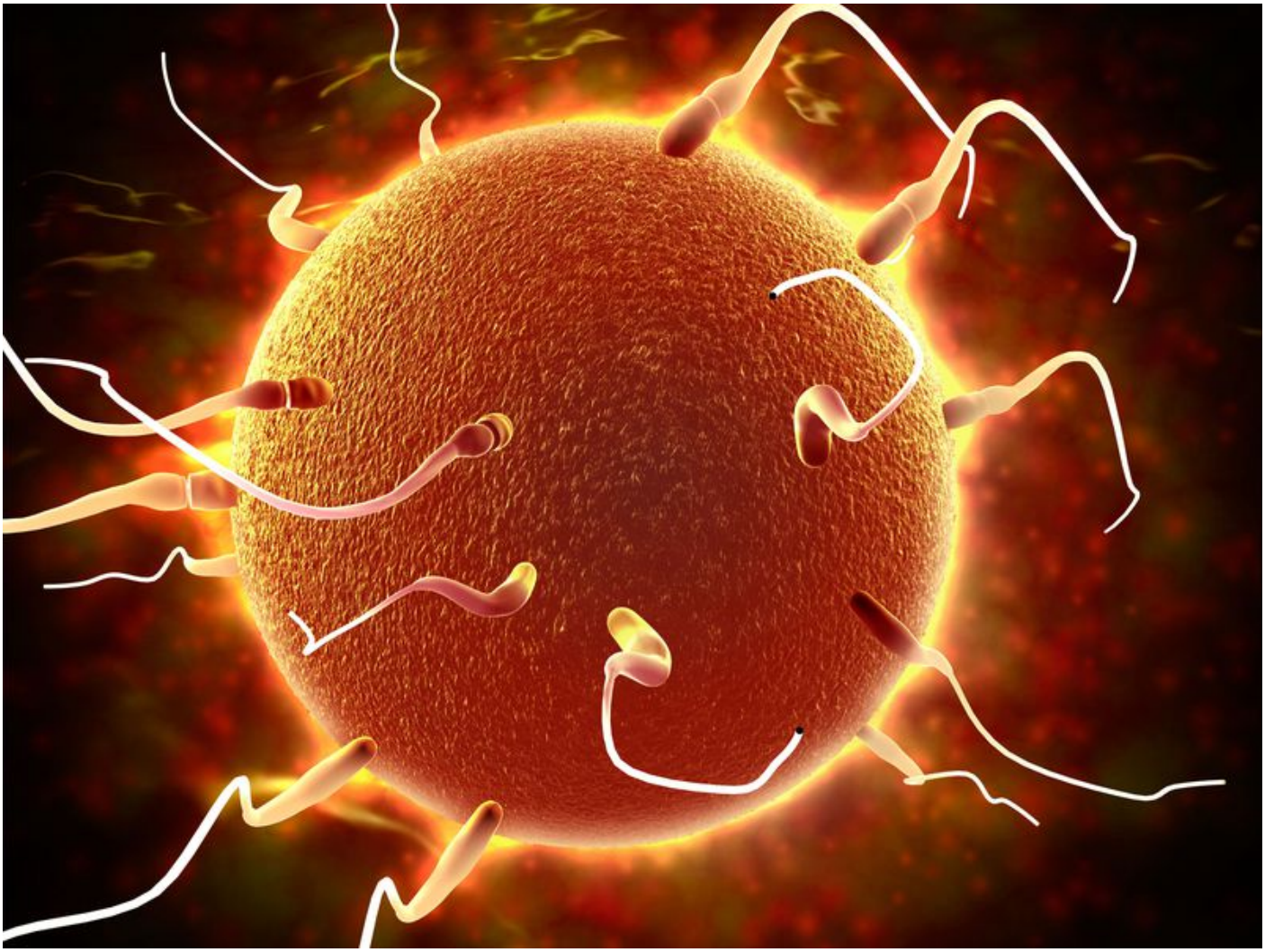
Dads Pass On More Than Genetics in Their Sperm

Seminal research reveals that sperm change their cargo as they travel the reproductive tract—and the differences can have consequences for fertility



For sperm, there's a vas deferens between start and finish, but the epididymis is what alters these swimmers en route. (Stocktrek Images, Inc./Alamy)

By Katherine J. Wu
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Eat poorly, and your body will remember—and possibly pass the consequences onto your kids. In the past several years, mounting evidence has shown that sperm can take note of a father's lifestyle decisions, and transfer this baggage to offspring. Today, in two complementary studies, scientists tell us how.

As sperm traverse the male reproductive system, they jettison and acquire non-genetic cargo that fundamentally alters sperm before ejaculation. These modifications not only communicate the father's current state of wellbeing, but can also have drastic consequences on the viability of future offspring.

Each year, over 76,000 children are born as a result of assisted reproduction techniques, the majority of which involve some type of in vitro fertilization (IVF). These procedures unite egg and sperm outside the human body, then transfer the resulting fertilized egg—the embryo—into a woman's uterus. Multiple variations on IVF exist, but in some cases that involve male infertility—for

instance, sperm that struggle to swim—sperm must be surgically extracted from the testes or epididymis, a lengthy, convoluted duct that cradles each testis.

After sperm are produced in the testes, they embark on a harrowing journey through the winding epididymis—which, in a human male, is about six meters long when unfurled—on their way to storage. Sperm wander the epididymis for about two weeks; only at the end of this path are they fully motile. Thus, while "mature" sperm can essentially be dumped on a waiting egg and be reasonably expected to achieve fertilization, sperm plucked from the testes and epididymis must be injected directly into the egg with a very fine needle. No matter the source of the sperm, these techniques have birthed healthy infants in four decades of successful procedures.

But scientists know genes are not the whole package. Over the course of a single lifetime, our genomes stay as they were originally written. However, how, when and why genetic instructions are followed can drastically differ without altering the manual itself—much like fiddling with the volume on a speaker without touching the wiring within. This phenomenon, called "epigenetics," helps explain why genetically identical individuals in similar environments, such as twins or laboratory mice, can still look and act in very different ways. And things like diet or stress are capable of cranking our genes' volume up and down.

One of the most powerful members of the epigenetic toolkit is a class of molecules called small RNAs. Small RNAs can conceal genetic information from the cellular machinery that carries out their instructions, effectively ghosting genes out of existence.

The legacy of a dad's behavior can even live on in his child if his epigenetic elements enter an embryo. For instance, mice born to fathers that experience stress can inherit the behavioral consequences of traumatic memories. Additionally, mouse dads with less-than-desirable diets can pass a wonky metabolism onto their kids.

Upasna Sharma and Colin Conine, both working under Oliver Rando, a professor of biochemistry at the University of Massachusetts Medical School, were some of the researchers to report such findings in 2016. In their work, Sharma and Conine noted that, in mice, while immature testicular sperm contain DNA identical to that of mature sperm, immature sperm relay different epigenetic information. It turns out that sperm small RNAs undergo post-testes turnover, picking up intel on the father's physical health (or lack thereof) after they're manufactured, but before they exit the body. However, the exact pit stop at which these additional small RNAs hitch a ride remained unknown.

To solve the mystery, Sharma, who led the first of the two new studies, decided to track the composition of small RNAs within mouse sperm as they fled the testes and cruised through the epididymis. She and her colleagues isolated sperm of several different ages from mice, including those about to emerge from the testes, those entering the early part of the epididymis and those in the late part of the epididymis. Sharma was surprised to find that many small RNAs seemed to be discarded or destroyed upon entering the early epididymis; then, the newly vacated sperm reacquired epigenetic intel that reflected the father's state of being, boasting a full set by the time they left the late epididymis.

There was only one possible source for the small RNA reacquisition: the cells of the epididymis—which meant that cells outside of the sperm were transmitting information into future generations.

"[The epididymis] is the least studied organ in the body," says Rando, who was senior author on both papers. "And it turns out this tube that no one ever thinks about plays a central role in reproduction."

To confirm that the epididymis was the culprit, Sharma's team added a chemical marker to a set of small RNAs in the epididymis and tracked their migration. As they suspected, tiny shipments of RNAs popped off of cells in the epididymis and fused with the sperm. Each stealthy swimmer then bore these epigenetic elements all the way to its final union with the egg.

It seemed that sperm at different points along the reproductive tract had the same genetics, but not the same epigenetics. Was this difference big enough to matter? Colin Conine, who led the second of the two new studies, next tested if using immature sperm would have noticeable effects on the offspring of mice. He and his colleagues extracted sperm from the testes, early epididymis and late epididymis and injected them into eggs. All three types of sperm were able to fertilize eggs. However, when Conine transferred the resulting embryos into mouse surrogates, none derived from early epididymal sperm—the intermediate stage devoid of most small RNAs—implanted in the uterus. The least and most mature sperm of the bunch were winners—but somehow, those in the middle were burning out, even though all their genes were intact.

This was baffling to all involved. "This intermediate broken stage was really stunning," says Rando.

At first, the researchers wondered if they had somehow isolated junky sperm doomed to be cleared from the early epididymis before reaching the ejaculate. But this didn't seem to be the case: all three types of sperm could fertilize eggs. The only other explanation was that the defect was temporary. If this was the case, then perhaps, if fed the right small RNAs, the early epididymal sperm could be rescued.

In her work, Sharma had noted that while the epigenetic cargo of testicular sperm and late epididymal sperm differ vastly, they had a few groups in common—but these small RNAs were evicted from sperm as they entered the epididymis, then reacquired from the cells along the meandering duct. Though bookended by success, the early epididymal flop was the only stage that lacked these elements—and the only stage incapable of generating an implantable embryo.

To test if these particular small RNAs were the key to fertility, the researchers pulled small RNAs out of the late epididymis and injected them into embryos fertilized with early epididymal sperm. To their amazement, these embryos not only implanted, but also yielded mouse pups—indistinguishable from embryos fertilized by late epididymal sperm. The early epididymal sperm was defective, but not irreversibly so. This hinted that the deficiency wasn't a fluke, but a normal part of the journey through the epididymal labyrinth. In other words, on the path to maturation, males were breaking sperm, then repairing the damage.

"It's very bizarre to see them lose [viability] and gain it back," says Sharma. And the utility of this back-and-forth remains entirely enigmatic. But whatever the reason, it's clear that sperm vary enormously along the length of the reproductive tract.

Mollie Manier, a professor who studies sperm genetics at George Washington University and was not affiliated with the study, praised the rigorous nature of this "very exciting" research. "These papers really add to our understanding of [how] dads can pass non-genetic information onto their kids," she explains. According to Heidi Fisher, a professor who studies sperm at the University of Maryland and also did not participate in the research, these "elegantly designed" experiments may also shed light on how problems with the epididymis could cause otherwise unexplained cases of male infertility.

In their future work, Rando's group will continue to study the mouse pups generated from sperm of various ages, keeping a close lookout for any long-term issues in their health. The team also hopes to pinpoint which small RNAs are directly responsible for successful implantation—and why sperm enter this bewildering period of incompetence.

"There's a lot of inheritance that we haven't yet explained," says Conine. "But animals are not just their DNA." However, Conine cautions that different doesn't always mean worse. Testicular and epididymal sperm from humans have helped, and continue to help, thousands around the world conceive children.

This comes with a small caveat. It wasn't until 1978 that the first baby was successfully born of an IVF procedure—and though thousands have followed since, this generation is still young. As of yet, there's no reason to suspect any negative consequences of in vitro versus natural conception; as this population ages, researchers will continue to keep close tabs. Since the majority of IVF procedures are performed with mature sperm that have cleared the late epididymis, Rando is not concerned.

And, in the unlikely case that there are repercussions to using testicular or epididymal sperm in these procedures, Rando remains hopeful that future work will enable scientists to restore the necessary information immature sperm might lack. Someday, addressing epigenetics may be key to enhancing assisted reproduction technology—and ensuring that sperm are as mature as they come.

About Katherine J. Wu

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