Researchers uncover defective sperm epigenome that leads to male infertility

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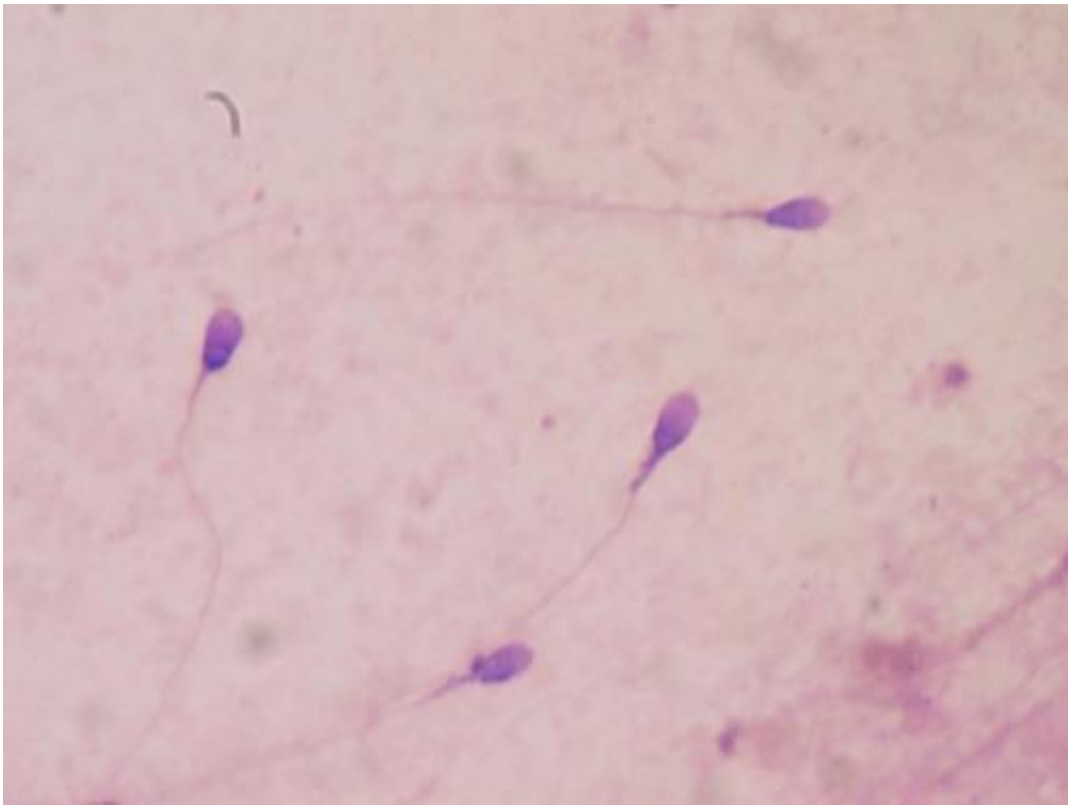

Human sperm stained for semen quality testing in the clinical laboratory. Credit: Bobjgalindo/Wikipedia

One out of eight couples has trouble conceiving, with nearly a quarter of those cases caused by unexplained male infertility. For the past decade, research has linked that infertility to defective sperm that fail to "evict" proteins called histones from DNA during development. However, the

mechanisms behind that eviction and where this is happening in the sperm DNA has remained both controversial and unclear.

Now, researchers at Penn Medicine show, using newer genome-wide DNA sequencing tools, the precise genetic locations of those retained histones, as well as a key gene regulating it. The findings were published in *Developmental Cell*.

Taking it a step further, the researchers created a new mouse model with a mutated version of the gene, Gcn5, which allows investigators to closely track the defects in sperm from the early stages of sperm development through fertilization and on. This is an important step forward as it could lead to a better understanding of not only infertility in men—and ways to potentially reverse it—but also the suspected epigenetic mutations being passed onto the embryo from males either naturally or through in vitro fertilization.

Epigenetics, the factors influencing an organism's genetics that are not encoded in the DNA, play a strong role in sperm and egg formation.

"For men who have unexplained infertility, everything may look normal at the doctors: normal semen counts, normal motility. Yet they can still have problems conceiving," said first author Lacey J. Luense, Ph.D., a research associate in the lab of study senior author, Shelley L. Berger, Ph.D., the Daniel S. Och University Professor in the departments of Cell and Developmental Biology and Biology, and director of the Penn Epigenetics Institute. "One explanation for persistent problems is histones being in the wrong location, which may affect sperm and then early development. Now, we have a really good model to study what happens when you don't get rid of the histones appropriately in the sperm and what that may look like in the embryo."

Healthy sperm lose 90 to 95 percent of histones, the main proteins in

chromatin that package DNA and turn genes on and off, and replace them with protamines, which are smaller proteins able to properly pack the DNA into tiny sperm. Given the role of retained histones in infertility and embryonic development, there is great interest in determining the genomic locations so they could potentially be utilized for further study and ultimately treatment.

Past studies have produced conflicting results on the whereabouts of histones. A technology known as MNase-sequencing that uses an enzymatic reaction to pinpoint location has placed the retained histones on important gene promotors. Other studies with the same approach found histones at DNA repeats and placed in so-called "gene deserts," where they play less of a role in regulation.

"There has been controversy in the field trying to understand these discrepant data," Luense said. "In this new study, we found that both of these previously described models are correct. We find histones on genes that appear to be important for embryo development, but we also find them at repetitive elements, places that do need to be turned off and to prevent expression of these regions in the embryo."

The researchers applied a technology known as ATAC-sequencing, a more precise and faster approach, to track waves of histones at unique sites across the genome during the early and late stages of sperm development in mice. ATAC-seq can identify parts of the genome open and closed—in this case, regions that retain the sperm histones—and then make a cut and tag the DNA, which can then be sequenced.

In the mouse models created with the mutated Gcn5 gene, the researchers found these mice to have very low fertility. The researchers also showed that retained histones in normal mice sperm correlated with histone positions in very early embryos, supporting the hypothesis that paternal histones transfer epigenetic information to the next generation.

Having this type of mutant model gives scientists a tool to closely study the mechanisms underlying the mutated sperm's trajectory and understand what effect it may have on the embryo and in development. It also opens an opportunity to study potential therapeutic targets.

"Right now, the burden of IVF and other assisted-reproductive technologies fall on women. Even it's the male factor, it's still women who have to go through hormone injections and procedures," Berger said. "Now imagine being able to apply epigenetic therapeutics to change the levels of histones and protamines in males before embryogenesis? That's one of the questions we want to explore and this model will allow us to move toward that direction."

There are numerous available epigenetic drugs used to treat cancer and other diseases. Given their mechanisms, treating sperm with drugs to increase histone eviction is one potential route to explore.

Limitations with human embryos in science have led to a lack of overall research on infertility and the role of the father's epigenome on embryo development, which underscores the importance of studies such as this, the researchers said.

"There are a lot different factors that can alter the sperm epigenome: diet, drugs, alcohol, for example," Luense said. "We are just now starting to understand how that can affect the child and affect development. These initial, basic studies that we are doing are critical, so we can better understand what's driving these epigenetic mutations."

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