“Insulin Dependent Diabetes Mellitus: Implications for Male Reproductive Function” (2007), by Ishola Agbaje, Deirdre Rogers, Carmel McVicar, Neil McClure, Albert Atkinson, Con Mallidis, and Sheena Lewis

In 2007, Ishola Agbaje, Deirdre Rogers, Carmel McVicar, Neil McClure, Albert Atkinson, Con Mallidis, and Sheena Lewis published “Insulin Dependent Diabetes Mellitus: Implications for Male Reproductive Function,” hereby “Diabetes Mellitus: Implications,” in the journal Human Reproduction. In their article, the authors explore the effects of elevated blood sugar in the form of diabetes mellitus on the quality of male sperm. When investigating possible fertility issues, fertility specialists often study semen, the male reproductive fluid that contains sperm cells to detect changes in sperm count, movement, and structure. In “Diabetes Mellitus: Implications,” the authors use both conventional semen analysis and technical molecular methods to assess the quality of sperm from diabetic and non-diabetic men. The authors found that men with diabetes had higher levels of DNA damage within their sperm and highlighted a need for additional research on the link between diabetes and male reproductive health.

At the time of the study’s publication in 2007, 11.2 percent of men in the United States older than twenty lived with diabetes. Agbaje, Rogers, McVicar, McClure, Atkinson, Mallidis, and Lewis theorized diabetes could cause damage to sperm DNA through oxidative stress, a process where oxygen radicals attack biological molecules like DNA, proteins, and fats. According to the Centers for Disease Control and Prevention, in 2007, about 2.6 percent of people between the ages of twenty and thirty-nine in the US had diabetes. Some research has suggested that sperm quality might decrease with age due to DNA damage caused by oxidative stress, which can result in sperm dysfunction and a decrease in fertility. In 2007, scientists had already established that diabetes can make oxidative stress worse, resulting in common complications of diabetes such as blindness, nerve damage, and heart diseases. However, researchers had not examined a possible relationship between diabetes and sperm DNA damage.

The authors of the study were based in Belfast, Northern Ireland, and affiliated with both the Regional Center for Endocrinology and Diabetes and the Regional Fertility Center. Agbaje, who led the research on “Diabetes Mellitus: Implications,” trained for his doctorate at the Queen’s University in Belfast, Northern Ireland, where he researched male infertility. The study’s co-authors, Rogers, McVicar, McClure, Atkinson, Mallidis, and Lewis, were part of the Belfast Reproductive Medicine research group. According to a United Kingdom news outlet, BioNews, Lewis stated that their study was small, but served to highlight a possible overlooked concern for men’s reproductive health.

The authors divided “Diabetes Mellitus: Implications” into four main sections. In the introductory section, the authors address the growing prevalence of diabetes and previous studies linking diabetes to adverse effects on male reproductive health. They also indicate the motivation behind their use of both conventional semen analysis and molecular techniques to compare the sperm quality of diabetic and non-diabetic men. In the next section, the authors describe that they performed a variety of analysis studies on the semen from fifty-six participants from Belfast, Northern Ireland. The authors explain the results of the semen analysis studies in the next section, stating that the diabetic group had slightly lower semen volume, and increased evidence of DNA damage. In the final section, the authors discuss the validity of using sperm DNA damage as biomarkers for fertility

issues. They also speculate a possible mechanism that could explain how diabetes might lead to damage of sperm DNA.

In the introductory section, the authors discuss the growing incidence of diabetes and assert that research should focus on the effect of diabetes on male fertility. The authors claim that diabetes is one of the greatest threats to global health, citing that the World Health Organization predicted that the number of people worldwide living with diabetes is projected to reach 300 million by 2025. In previous studies, researchers had already linked diabetes to generalized sexual dysfunction, although the extent of sperm DNA damage in that dysfunction remained unclear. Because diabetes causes high blood sugar and can damage nerves and blood vessels, diabetic men can have difficulty achieving an erection or ejaculating. Studies also suggested that diabetic men experienced higher rates of infertility and contributed to pregnancies with higher rates of miscarriage.

Despite the results of previous studies, in 2007 there was relatively limited research that examined the effect of diabetes on human sperm quality. To make up for that, the authors compared the sperm of diabetic and non-diabetic men using both conventional and molecular techniques. If diabetes, which affects more men than women, does cause genetic damage to sperm, theoretically scientists would be able to see that damage using molecular techniques. When DNA, a double-stranded structure containing genetic information, becomes damaged through fragmentation or deletions, that damage gives rise to mutations, which sometimes cause health issues like cancer. During fertilization, the DNA in egg cells can repair some DNA damage in sperm cells to a certain degree, but if damage persists after fertilization, there may be higher rates of miscarriage and the later development of cancers like leukemia.

In their methods section, Agbaje, Rogers, McVicar, McClure, Atkinson, Mallidis, and Lewis describe how they obtained participants and collected their sperm before analyzing it using conventional semen analyses, Comet assays, and Long-PCR. They recruited twenty-seven men with Type-1 diabetes, a type of diabetes where the body is incapable of producing enough insulin to regulate blood sugar, from the Royal Victoria Hospital in Belfast, Northern Ireland. The researchers also enrolled twenty-nine non-diabetic men from the Royal Maternity Hospital in Belfast, Northern Ireland, as a control group. After two to five days of sexual abstinence, each man provided a semen sample that a lab technician immediately incubated and analyzed for volume, movement, structure, and count. The lab technician used the remaining semen for the additional molecular tests.

The researchers used both Comet assay and Long-PCR to conduct the molecular tests on the sperm samples. Cancer researchers use the Comet assay, or single cell gel electrophoresis, to detect DNA fragmentation within a cell's nucleus, a pouch that contains tightly folded DNA. Because the Comet assay separates nuclear DNA, or nDNA, by size, if there is fragmentation, the resulting image on the gel will look like a comet. Researchers can test for additional damage like DNA deletions within mitochondria, a membrane-bound sac that produces energy for the cell, using long-range polymerase chain reaction, or Long-PCR. Additionally, other studies had associated those deletions with impaired sperm movement and infertility. Therefore, if diabetes was proven to cause sperm DNA damage, there could be significant negative effects on male fertility. To conduct the Comet assay, Agbaje, Rogers, McVicar, McClure, Atkinson, Mallidis, and Lewis first embedded the sperm in agarose gel, which served as the medium for the test. Then the researchers freed the nDNA from the sperm before testing it for fragmentation possibly caused by diabetes. To then look for mtDNA deletions possibly caused by diabetes, researchers isolated mtDNA from the sperm cells and multiplied it to a testable amount using Long-PCR.

Agbaje, Rogers, McVicar, McClure, Atkinson, Mallidis, and Lewis found that, in comparison with the control group, sperm from the diabetic men had normal concentration, movement, and shape, but higher levels of nDNA fragmentation and mtDNA deletions. Under the World Health Organization's general standards for conventional semen analysis, the diabetic men had normal semen profiles with only one exception. The diabetic men had a slightly smaller volume of semen than non-diabetic men. However, the results from the molecular techniques showed a much larger difference between the control group and diabetic men. The average percentage of fragmented sperm nDNA was thirty-two percent in the control group and fifty-three percent in the diabetic group, which the authors assert may suggest that diabetes is linked with increased sperm DNA fragmentation. Although the size of the mtDNA deletions did not differ significantly between both groups, men in the diabetic

group had an average of four mtDNA deletions in comparison to men in the control group, who had an average of three. In some specific cases, one mtDNA deletion may cause certain life-threatening syndromes.

In their article's discussion, Agbaje and colleagues explore the general relationship between sperm genetic integrity and male fertility and examine why diabetes could lead to sperm damage. They referenced one study conducted in 2002 by Ian Morris and colleagues at St. Mary's Hospital in Manchester, England, where the researchers found that nDNA damage detected by Comet assay was predictive of failed embryonic development in assisted reproductive technologies. Agbaje, Rogers, McVicar, McClure, Atkinson, Mallidis, and Lewis state that they also found that men who smoke experience increased levels of sperm DNA damage, and produce children who are more likely to suffer from childhood cancers such as leukemia and lymphoma. They reiterate that elevated sperm DNA damage decreases fertility and hypothesize that diabetes can be responsible for similar damage due to the oxidative stress it can cause in cells. Oxidative stress happens when there is an imbalance between unstable oxygen free radicals, molecules with one or more unpaired electrons, and antioxidants, substances that neutralize free radicals.

Since the authors published "Diabetes Mellitus: Implications" in 2007, researchers from other institutions have further examined the relationship between diabetes and oxidative stress. Some of that research has focused on advanced glycation end-products, or AGE, which are proteins or fats that have been glycated, or bonded to sugar. Men with diabetes have higher levels of AGEs in their reproductive tracts, and AGEs have been shown to trigger oxidative stress in cells. In other words, it is possible that the presence of AGEs in excessive amounts can become harmful to sperm DNA, potentially leading to issues with fertility. Animal-derived foods high in protein and fat tend to be AGE-rich, while foods high in carbohydrates like fruits, vegetables, and whole grains tend to have low amounts of AGEs. However, it is unclear how much of a role diet plays in heightened amounts of AGEs in those with diabetes. Research has also found that increased levels of blood sugar create AGEs within the body itself. The authors of "Diabetes Mellitus: Implications" suggest that additional studies will help pinpoint why diabetes causes oxidative stress, as well as the extent of diabetes' role in sperm DNA damage.

Sources

1. Agbaje, Ishola, Deirdre Rogers, Carmel McVicar, Neil McClure, Albert Atkinson, Con Mallidis, and Sheena Lewis. "Insulin Dependent Diabetes Mellitus: Implications for Male Reproductive Function." Human Reproduction 22 (2007): 1871–7. https://doi.org/10.1093/humrep/dem077 (Accessed July 21, 2020).
2. Asmat, Ullah, Khan Abad, and Khan Ismail. "Diabetes Mellitus and Oxidative Stress–A Concise Review." Saudi Pharmaceutical Journal 24 (2016): 547–53. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5059829/ (Accessed July 21, 2020).
3. Brookshire, Bethany. "Explainer: Prokaryotes and Eukaryotes." Science News for Students. https://www.sciencenewsforstudents.org/article/explainer-prokaryotes-and-eukaryotes (Accessed July 21, 2020).
4. Gahl, William. "Mitochondrial DNA." Genome.gov. https://www.genome.gov/genetics-glossary/Mitochondrial-DNA (Accessed July 21, 2020).
5. Harris, Isiah D., Carolyn Fronczak, Lauren Roth, and Randall B. Meacham. "Fertility and the Aging Male." Reviews in Urology 13 (2011): 184–90. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3253726/ (Accessed July 21, 2020).
6. Healthy Male Andrology Australia. "Diabetes and Reproductive Health." Australian Government Department of Health. https://www.healthymale.org.au/mens-health/diabetes-and-reproductive-health (Accessed July 21, 2020).
7. "Ishola Michael Agbaje." ResearchGate. https://www.researchgate.net/profile/Ishola_Agbaje (Accessed July 21, 2020).
8. Langie, Sabine, Amaya Azqueta, and Andrew R. Collins. "The Comet Assay: Past, Present, and Future." Frontiers in Genetics, 2015. https://www.frontiersin.org/articles/10.3389/fgene.2015.00266/full (Accessed July 21, 2020).

9. Mayo Clinic. “Diabetes.” Mayo Foundation for Medical Education and Research, August 8, 2018. https://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444 (Accessed July 21, 2020).
10. Morris, Ian D. “The Spectrum of DNA Damage in Human Sperm Assessed by Single Cell Gel Electrophoresis (Comet Assay) and Its Relationship to Fertilization and Embryo Development.” Human Reproduction 17 (2002): 990–8. https://doi.org/10.1093/humrep/17.4.990 (Accessed July 21, 2020).
11. “National Diabetes Fact Sheet, 2007.” Centers for Disease Control and Prevention. https://secure.in.gov/isdh/files/CDC_NDFS2007.pdf (Accessed July 21, 2020).
12. Sinclair, Katy. “Diabetes Linked to Male Infertility.” BioNews, 2007. https://www.bionews.org.uk/page_90358 (Accessed July 21, 2020).
13. Uribarri, Jaime, Sandra Woodruff, Susan Goodman, Weijing Cai, Xue Chen, Renata Pyzik, Angie Yong, Gary E Striker, and Helen Vlassara. “Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet.” Journal of the American Dietetic Association 110 (2010): 911–6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3704564/ (Accessed July 21, 2020).
14. Vignera, Sandro La, Rosita Condorelli, Enzo Vicari, Rosario D'Agata, and Aldo E. Calogero. “Diabetes Mellitus and Sperm Parameters.” Journal of Andrology 33 (2012): 145–53. https://onlinelibrary.wiley.com/doi/full/10.2164/jandrol.111.013193 (Accessed July 21, 2020).
15. Vlassara, Helen, and Jaime Uribarri. “Advanced Glycation End Products (AGE) and Diabetes: Cause, Effect, or Both?” Current Diabetes Reports 14 (2014): 453. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3903318/ (Accessed July 21, 2020).