AN INTERNATIONAL RESEARCH TEAM based at Wayne State University is developing a simple, non-invasive screening test that will not only determine whether a man is infertile, but will reveal the cause of that individual’s infertility. On a broader scale, the work may answer the widely publicized question of whether the general male population in the United States and other western nations is actually experiencing a decline in fertility, and if so, identify probable culprits as well as solutions.

For couples who are having difficulty starting a family, the news means that men may soon be able to skip the extensive battery of tests, including a testicular biopsy, that is currently part of the infertility screening. Instead, they may only need to provide a sperm sample. In addition, the research team believes the test may provide enough specific information so that medical professionals can better direct treatments, or can identify which couples will never conceive. The latter diagnosis will allow couples to forgo the lengthy, expensive, and ultimately futile infertility therapy, and begin considering other options, such as sperm donors.

This promising new test will likely have a broad reach, remarked one of the team members. “Infertility involves about one out of every six couples who are trying to conceive, so it is actually a huge clinical problem that many couples face,” said Michael Diamond, MD, the Kamran S. Moghissi Professor and associate chairman of the WSU obstetrics and gynecology department. He added that most infertile men actually produce sperm, but for some reason, those sperm are unable to fertilize an egg. “If this new technique does all of the things we’re hoping and expecting it to do, it could change the way male-factor infertility is diagnosed around the country and around the world.”

Beyond that, he added, “It has all sorts of potential when you start looking at what effects pharmaceutical drugs have on sperm, sperm function and other sperm characteristics. It can also possibly establish the effects of environmental toxins, alcohol, caffeine, smoking or illicit drugs on sperm. So this work may have extensive impacts.”

Microarrays and mRNA Used in Male Fertility and Reproductive Health

BY LESLIE MERTZ

Microarrays like this are helping to identify genes associated with male infertility.
More Than Just DNA

The test grew from a finding made simultaneously in two labs on an ocean apart: Stephen Krawetz at Wayne State and David Miller at the University of Leeds in the U.K. separately verified that male sperm conferred something other than DNA during fertilization. These male reproductive cells also carried mRNA, or the genetic message with the blueprint for making the sperm. Upon learning of his research, Krawetz contacted Miller, and the two started working together. Miller soon decided to spend time in Krawetz’s lab, and is currently at Wayne State pursuing their investigation.

“We thought that if we could analyze that message in the mRNAs, and compare an infertile man with men who are normally fertile — those who have fathered children and see differences, then we would get a closer understanding of what causes that man’s infertility,” said Miller, PhD, associate professor in the department of obstetrics and gynecology at Leeds. “In other words, we feel that the mRNA message is crucial to understanding what’s gone wrong.”

The collaboration didn’t end there. “At the same time, I met David Dix of the Environmental Protection Agency,” said Krawetz, PhD, of the WSU obstetrics and gynecology department, and the university’s Center for Molecular Medicine and Genetics (CMMG) and Institute for Scientific Computing. “We put together this research triangle between Wayne State, the EPA and the University of Leeds, and through that, I was invited to be one of the founding members of the EPA Microarray Consortium (http://www.epa.gov/nheerl/epamac). This partnership enabled us to produce arrays that trap specific mRNAs. When sperm is added, color changes at each trap site indicate whether the sperm includes a specific mRNA. Almost immediately, the researchers can scan the sperm to tell which mRNA, and which associated genes, are present. They already have a basic profile of a fertile male, and are refining it now.

Next, they will begin to study infertile men. Using the same technology, they will add infertile sperm to the microarrays to compare it against the fertile-male profile. They will then document the points of difference, run those discrepancies through an algorithm they are developing with help from the WSU computer science department, and deduce the causes of male-factor infertility.

“We would ‘spot’ or add onto the microarray all the genes that we find are associated with male factor infertility,” Dr. Miller described. “Then it would be a machine, basically, that would read off and give the clinician some idea of a) what the underlying causes are, and b) what options the patient has for treatment or management of his infertility.”

Dr. Krawetz added, “On the basis of this work, we believe we will be able to devise a preclinical screen for men or couples who are seeking infertility counseling. We should be able to detect a certain percentage of the population who are infertile based on their patterns of mRNA.”

Environmental Health

This work may also have ramifications for male reproductive health in general, the research team asserts. “We feel this is going to be quite important in the next few years in not just unraveling what causes a man’s infertility, but also in helping us to understand whether there are reductions in sperm counts occurring in men in the West. If that’s the case, we might get some handle on what’s causing that too,” Miller said, explaining that the normal-male profile will again provide the basis for the comparison.

“We now have a window into the male reproductive system in which the mRNA message is crucial to understanding the cause of that man’s infertility,” said Miller, explaining that the normal-male profile will again provide the basis for the comparison. “This work may also have ramifications for male reproductive health in general, the research team asserts. “We feel this is going to be quite important in the next few years in not just unraveling what causes a man’s infertility, but also in helping us to understand whether there are reductions in sperm counts occurring in men in the West. If that’s the case, we might get some handle on what’s causing that too,” Miller said, explaining that the normal-male profile will again provide the basis for the comparison.

“Over the last 10 years, there has been an increasing amount of evidence that the effects of environmental exposures on human reproductive health,” reported team member David Dix, PhD, a research biologist and microarray specialist at the EPA. He cited the recent media coverage of endocrine-disrupting chemicals and their possible connection to male fertility as one example. With the new normal-male profile and microarrays, Dr. Dix said, “we now have a window into the male reproductive system in which we can monitor overall reproductive health with precision. In fact, we can get tens of thousands of different questions answered from a single experiment — not only predicting for us whether this individual will be fertile or not, but giving us a detailed description of the gene-environment interaction for that individual.” In the latter case, microarrays would target genetic differences between the normal-male model and men who have been exposed to suspected toxins. “You can create a whole series of tools, or perhaps one very large microarray tool, that would allow you to monitor the potential effects of environmental chemicals on male reproductive health,” Dr. Dix said.

The microarray test would give a man a quick determination of whether his sperm had been adversely affected by a toxin. “That approach would take into account not only how many people respond and the exposure limits that have been set on how most people seem to respond, but how each individual responds,” he said, pointing out that different men have different genetic susceptibilities. “We would ‘spot’ or add onto the microarray all the genes that we find are associated with male factor infertility,” Dr. Miller described. “Then it would be a machine, basically, that would read off and give the clinician some idea of a) what the underlying causes are, and b) what options the patient has for treatment or management of his infertility.”

Dr. Krawetz noted, “This is the future of testing and patient care.”

Patients and Potential

In addition, their research may be a boon to animal husbandry, he continued. “Beef production is a good example. Cattle have a long gestation time, so if you could ensure that every pregnancy outcome was successful, you could increase your beef production, and the profit margin would then go up considerably.”

Dr. Miller added, “It can be applied to any animal of economic value to help improve the breeding stock. We know that most other animals that have been studied have mRNA in their sperm, too, so it wouldn’t be difficult to extend this to examine the spermatozoa of those animals.”

Buoyed by their success and the great potential of this work, the researchers have jointly filed provisional-patent applications on the technology, and anticipate full patents by the end of 2002. Mostly, however, they look forward to the research yet to come. “This shows that international collaboration is essential to furthering successful science,” Dr. Miller remarked. “It also shows that there is room still for communication beyond the bounds of the United States and vice versa, and it is going to be for the common good.”

Added Dr. Diamond, “We’ve heard so much about the Human Genome Project. At least on the OB/GYN side, this will be one of the original approaches that actually takes some of that information about all of the genes that have been identified and brings it to the practice of medicine. This is the type of thing that can come from these sorts of investments in the future.”