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By [Monica Heger](#)

Two research groups, one from the University of Oxford and another from Harvard University, are developing single-cell sequencing-based tests designed to improve the success rates of *in vitro* fertilization procedures by screening embryos for chromosomal abnormalities before they are implanted.

Dagan Wells from the University of Oxford's Nuffield department of obstetrics and Xiaoliang Sunney Xie from Harvard University discussed slightly different single-cell sequencing techniques at last week's Advances in Genome Biology and Technology meeting in Marco Island, Fla.

Both groups have demonstrated in proof-of-concept studies that the tests can accurately detect chromosomal aneuploidy in clinically relevant time frames and are now starting clinical trials to compare their methods to the standard of care for screening embryos prior to implantation.

Other studies, based on microarrays, have found that genetic screening of embryos could improve the success rates of IVF procedures by as much as 50 percent, Wells told *Clinical Sequencing News*.

Nearly 10 percent of all couples have fertility issues, and around 5 million babies have been born as a result of assisted reproductive technology, Wells said. However, the actual success rate is still very low with only 25 percent to 30 percent of all IVF cycles resulting in a baby being born.

One main reason for this inefficiency is that methods for choosing which of several fertilized eggs to implant in the womb are not precise. Typically, physicians do not want to transfer more than one embryo because there is the risk not only of multiple births, but also of preeclampsia

and maternal hemorrhage, Wells said.

Instead, the embryo is selected based on its morphology, "which we know now is a poor guide to embryo viability," he said.

The primary reason for embryos not being viable is due to chromosomal abnormalities, Wells said. As such, Wells' team at the University of Oxford, in collaboration with Reprogenetics, a New Jersey-based firm that specializes in preimplantation genetic diagnosis, has designed a sequencing-based test that uses whole-genome shotgun sequencing of a single cell taken from the eight-cell blastomere stage of an embryo to identify chromosomal aneuploidies.

The test is very similar to the strategies employed by companies like Sequenom and Verinata in their prenatal trisomy tests, except that in Wells' case, he is applying it to single cells.

The test uses Life Technologies' Ion Torrent PGM to identify chromosomal aneuploidies, a main cause of IVF failure. Wells' team has already validated the assay against known samples with a 100 percent concordance rate, and has begun testing the assay in some clinical samples and plans to begin a randomized clinical trial of the test.

The cell used in the assay is obtained by dissolving the thin membrane surrounding the blastomere and carefully plucking one cell from the bunch. The rest of the complex is then sent back to the incubator where it continues to develop. After DNA is extracted, the genome is amplified via multiple displacement amplification. Then the DNA is shotgun sequenced on the PGM. Wells said the group settled on the PGM primarily because of its fast turnaround time and low cost.

With IVF, there is a limited window of time in which a genetic test can be done on the embryo, he said. After the egg is fertilized, it is grown for three days, after which one cell is removed and tested. But the embryo must be transferred to the womb no later than five or six days after fertilization, Wells said, leaving around 24 hours to determine whether it is viable or not.

"After five or six days, the endometrium will no longer be receptive to the embryo, and the embryo may have gone beyond the point in development where it's able to implant," he said.

In preliminary work, Wells' team has been able to complete the test accurately in 12.5 hours for an estimated cost of \$70 when 32 samples are multiplexed.

Wells said that he is using the 316 chip on the PGM with 200 base paired reads, and generating around 2.5 million reads per run, or around 77,000 reads per sample. While this corresponds to very low depth of coverage, that is all that is needed to determine chromosomal aneuploidy, he said.

One problem that has plagued single-cell sequencing techniques is the significant bias from the amplification step. Wells said that while MDA does result in bias — for instance chromosome 19 has an overabundance of reads mapping to it despite being a short chromosome — Wells said that in their validation studies, the researchers found that the bias could be accounted for. The number of reads per chromosome is reproducible, he said, so aneuploidy can be determined by comparing the number of expected reads to the number of observed reads.

Already, Wells' group has validated the test against 50 known samples, including 10 from known aneuploidies and 40 embryos that had been previously diagnosed with array CGH.

All 50 samples yielded a result, with the 48 aneuploidies correctly called.

However, Wells said that he has found some cases where the test does not produce a result due to the DNA being unable to amplify. "In those cases, it may be because the cell was apoptotic," he said, "But in all cases where there's amplification, we get concordant results."

The team has now moved on to testing clinical samples, and has so far tested around 20 samples. The researchers are also setting up a clinical trial to determine whether the sequencing-based test can improve pregnancy rates over the standard morphology-based testing.

Other studies, based on array CGH, suggest that genetic testing of embryos could improve pregnancy rates by as much as 50 percent, Wells said.

Wells said that there are currently also a number of clinical trials for array CGH-based genetic tests, but he said sequencing-based tests may offer several advantages in terms of cost, speed, and the amount of information that can be gleaned from the test itself.

At around \$70, the sequencing-based test is already about half the cost of a microarray-based test, Wells said. And, because women undergoing IVF will often produce multiple oocytes in a cycle, or go through more than one IVF cycle, those costs can quickly add up.

Wells expects to begin the clinical trial of the shotgun sequencing approach for detecting chromosomal abnormalities in another couple of months with results available by the end of the year. For the trial, he plans to test 100 patients and compare the sequencing test to the standard-of-care morphology-based approach.

Harvard's Sunney Xie's test is also based on whole-genome shotgun sequencing of a single cell, but uses a slightly different method. Rather than MDA, he uses a whole-genome amplification method that his team published last year in *Science*, called multiple annealing and looping-based amplification cycles, or MALBAC ([*IS 1/2/2013*](#)).

Xie said that MALBAC has an advantage over MDA in that it results in less bias because it is based on linear amplification, rather than exponential amplification.

Additionally, he is testing the technique on two different stages in embryonic development. Like Wells' team, he is looking at one cell from the eight-cell blastomere, but he is also testing it on the first and second polar bodies.

Polar body cells are produced in the meiosis cycle, with the first body containing the maternal diploid genome and the second polar body containing the maternal haploid genome. By sequencing both polar bodies, the maternal genome of the embryo can be deduced.

Sequencing the polar bodies rather than one of the cells from the blastomere has the advantage in that the polar body cells are not contained within the blastomere, so there is no risk in harming a potentially viable embryo.

Additionally, the polar bodies can be removed earlier in the IVF cycle, thus leaving a two- to three-day window in which to perform the test, compared to only one day when screening a cell from the blastomere.

In a proof-of-concept collaboration with a group led by Jie Qiao from Peking University Third Hospital and including Fuchou Tang from Peking University's Biodynamic Optical Imaging Center, Xie tested the technique on six female donors that had already had children, showing that the technique could correctly infer embryo aneuploidy by sequencing the two polar bodies to 0.1-fold depth.

Now, Qiao, whose group at Third Hospital sees 10,000 IVF patients per year, is planning a clinical trial of the technique. Qiao will initially enroll 30 patients in the trial, testing both the polar bodies and one blastomere cell from each patient. If the results are good, the trial will expand to an additional 100 patients, followed by another 1,000 patients.

The sequencing technique will be compared to morphology-based testing. The hospital also has data from SNP arrays and array CGH, which it has tested in the genetic screening of embryos, and success rates from the MALBAC-based test can also be compared to those, Xie said.

Xie declined to disclose what sequencing platform would be used in the clinical trial.

Single-Gene Disorders and Mitochondrial DNA

Such single-cell tests could also be tweaked to look for carrier status of certain genes — another advantage they have over microarrays, Wells said. While the depth of sequencing is too low to detect point mutations, an additional PCR enrichment could be done to "spike in" a gene of interest, he said.

For example, said Wells, if both individuals of a couple are carriers for cystic fibrosis, they will want to ensure that the implanted embryo does not have cystic fibrosis. In this case, following whole-genome amplification, an additional PCR enrichment can be done targeting the CF gene, so that the gene will have increased coverage compared to the rest of the genome, and disease-causing mutations can be identified.

Mitochondrial disorders can also be identified, Wells said, because blastomeres contain many copies of the mitochondrial genome. Testing a cell from a cell line with a known mitochondrial disorder, Wells' team showed the method could detect the disease-causing mutation. The researchers were also able to quantify the proportion of mitochondrial DNA that was affected by the mutation.

Additionally, Wells said that when the team compared the quantity of mitochondrial DNA among various normal cells and cells with chromosomal aneuploidy, they found that cells with chromosomal aneuploidy also had more mitochondria.

While more research needs to be done in determining whether and how chromosomal aneuploidy and mitochondria quantity are related, Wells said that evaluating the mitochondrial genome could provide another layer of screening that could improve IVF success.

While array-based studies have found that screening embryos for chromosomal aneuploidies

could improve IVF success rates to as much as 80 percent, there is still a significant failure rate and "one challenge is to find out what explains that other percentage," Wells said.

"One of the prime candidates is the mitochondrial genome, and this is data that we get when we [sequence] the embryo," Wells said.

The additional mitochondrial information "could help decide between two chromosomally normal embryos," Wells said.

The mitochondrial DNA testing and disease gene testing are still in the research phase, Wells said. In the meantime, he said, as the team gears up for the clinical trial of its aneuploidy test, it is working to further optimize it, including reducing the number of steps involved and decreasing turnaround time.

Aside from showing clinical utility, Wells said that an economic analysis would also have to be done before the test is likely to be adopted. "It will come down to an issue of health economics. How much is it worth to the patients, the health care system, the insurers?" he said.

"Given the advantages we've seen and the clear benefits to patients not having to undergo multiple IVF cycles, or miscarriages, or termination of wanted pregnancies [due to serious abnormalities], ... all of those things add up to a huge value not only to the patient, but the healthcare system and insurance provider. It could potentially represent huge savings."



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